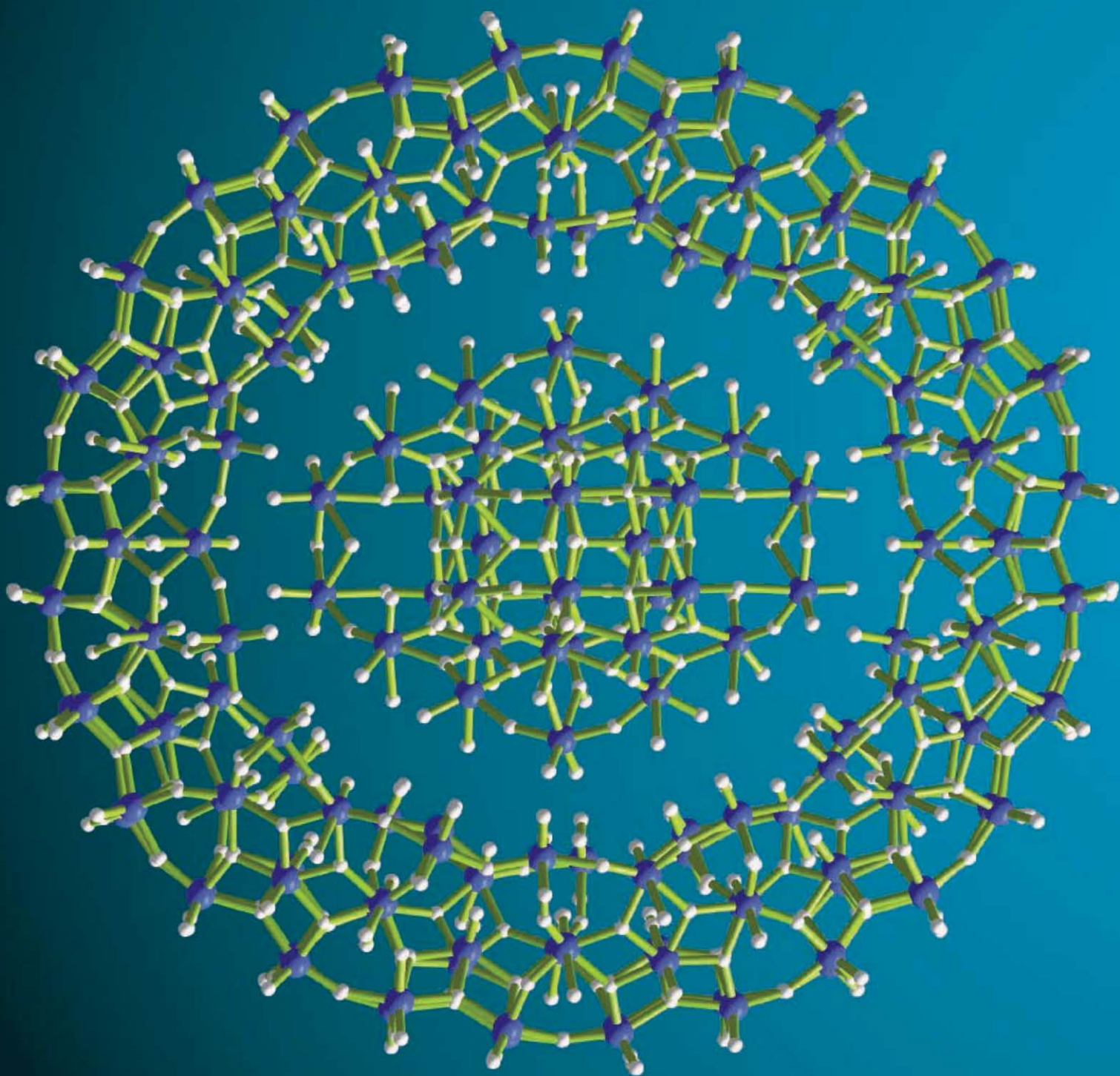


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TNF-beta, human
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A wide-angle photograph of the San Diego skyline, featuring numerous high-rise buildings and skyscrapers along the waterfront. The sky is blue with scattered white clouds. The water in the foreground is calm and reflects the city.

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- ▶ And celebrate one of the greatest inventions of the 20th century — the laser

President's Address



Peter C. Agre, M.D.

AAAS President, and Director, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health

Agre shared the 2003 Nobel Prize in Chemistry with Roderick MacKinnon of Rockefeller University for the discovery of aquaporins, the key proteins that transport water across cell membranes.

Not long after receiving the Nobel Prize, Agre began working to extend his studies of aquaporins to malaria, addressing the question of whether or not aquaporins could be exploited as a means of treating or preventing the disease. Initial results led his laboratory to focus on malaria as its primary area of study. As director of the Malaria Research Center, he oversees 19 Hopkins faculty members who concentrate on advancing basic science to develop new methods in malaria prevention and treatment. Agre is a member of the National Academy of Sciences (NAS), chair of the NAS Committee on Human Rights, and a Fellow of AAAS and the American Academy of Arts and Sciences. He received his M.D. degree from Johns Hopkins University.

Plenary Speakers



Carol W. Greider, Ph.D.

Daniel Nathans Professor and Director, Department of Molecular Biology and Genetics, and Professor of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Title To Be Determined

Greider, one of the world's pioneering researchers on the structure of telomeres, was awarded the 2009 Nobel Prize in physiology or medicine by the Royal Swedish Academy of Sciences along with Elizabeth Blackburn and Jack W. Szostak. While a 23-year-old graduate student at the University of California, Berkeley, working together with Blackburn, Greider discovered the enzyme telomerase and later, in her own lab, she cloned its RNA component. This work laid the foundation for studies that have linked telomerase and telomeres to human cancer



and age-related degenerative disease. It represents another example of curiosity-driven basic research that has direct medical implications. Greider obtained her Ph.D. degree in molecular biology from UC Berkeley in 1987.



Eric S. Lander, Ph.D.

Director, The Broad Institute of MIT and Harvard University, and Co-Chair, President's Council of Advisors on Science and Technology (PCAST) *Science and Technology in the First Year of the New Administration*

Lander is widely known as one of the driving forces behind today's revolution

in genomics, the study of all of the genes in an organism and how they function together in health and disease. He also is co-chair of President Obama's council of science and technology advisers. PCAST is an advisory group of the nation's leading scientists and engineers who directly advise the President and make policy recommendations in the many areas where understanding of science, technology, and innovation is key to strengthening the economy and forming policy. Lander also was one of the principal leaders of the Human Genome Project and is a member of both the National Academy of Sciences and Institute of Medicine. He is also an AAAS Fellow. Lander earned his B.A. degree in mathematics from Princeton University and Ph.D. degree in mathematics from Oxford University as a Rhodes Scholar.



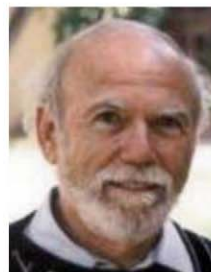
Marcia McNutt, Ph.D.

Director, U.S. Geological Survey, and Science Adviser to the Secretary, U.S. Department of the Interior (*invited*) *Science Below the Sea*

McNutt's appointment in 2009 marked a milestone for USGS — she is the first female director in the agency's 130-year history. She heads a multi-

disciplinary organization that focuses on biology, geography, geology, geospatial information, and water, and is dedicated to studying the landscape, natural resources, and natural hazards. Most recently she served as president and chief

executive officer of the Monterey Bay Aquarium Research Institute. Her biography includes a broad range of research interests and numerous honors and awards. Her research has ranged from studies of ocean island volcanism in French Polynesia to continental break-up in the Western United States to uplift of the Tibet Plateau. She also spent 3 years with the USGS in California working on earthquake prediction. She is a member of the National Academy of Sciences and a Fellow of AAAS. McNutt earned her Ph.D. degree in earth sciences at the Scripps Institution of Oceanography.



Barry C. Barish, Ph.D.

Director, Global Design Effort for the International Linear Collider (ILC), and Linde Professor of Physics, emeritus, California Institute of Technology, Pasadena

Lecture Title To Be Determined

Among Barish's noteworthy experiments were those performed at Fermilab using high-energy neutrino collisions. These experiments were among the first to observe the weak neutral current, a linchpin of electroweak unification theories. Today he directs the ILC, the highest priority future project for particle physics worldwide that promises to complement the Large Hadron Collider at CERN in exploring the TeV energy scale. In the 1980s, Barish initiated an ambitious international effort to build a sophisticated underground detector which provided some key evidence that neutrinos have mass. In 1994, he became principal investigator of the Laser Interferometer Gravitational-Wave Observatory (LIGO) project. As director of the LIGO Laboratory from 1997 to 2005, he led a team of scientists who built two facilities to detect and study gravitational waves from astrophysical sources. Barish is a member of the National Academy of Sciences and is a Fellow of AAAS. He earned his Ph.D. degree in experimental high energy physics at the University of California, Berkeley.

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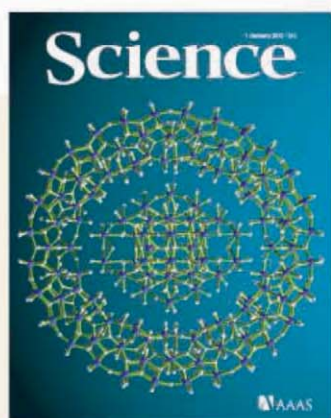
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COVER

Crystal structure of a molybdenum oxide nanowheel, 2.6 nanometers in diameter, around a smaller molybdenum oxide cluster. *Miras et al.* (page 72; related Perspective page 38) used a controlled-flow reactor to show that the central core serves as a transient template for the self-assembly of the nanowheel and is ultimately ejected to yield a hollow finished product.

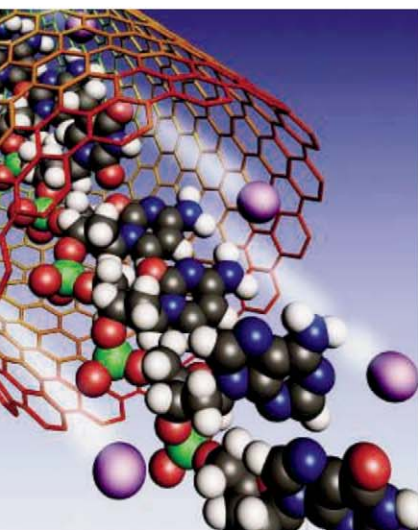
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Detection of Gamma-Ray Emission from the Vela Pulsar Wind Nebula with AGILE

A. Pellizzoni et al.

Pulsar wind nebulae could account for some of the yet unidentified Galactic gamma-ray sources.
10.1126/science.1183844

$^{238}\text{U}/^{235}\text{U}$ Variations in Meteorites: Extant ^{247}Cm and Implications for Pb-Pb Dating

G. A. Brennecka et al.

Variable abundances of meteorite isotopes may require correcting the lead-based age of the solar system by 5 million years.
10.1126/science.1180871

Darwinian Evolution of Prions in Cell Culture

J. Li et al.

When propagated in vitro, prion strains demonstrate adaptability and selection.
10.1126/science.1183218

Protein PRDM9 Is a Major Determinant of Meiotic Recombination Hotspots in Humans and Mice

F. Baudat et al.

10.1126/science.1183439

Drive Against Hotspot Motifs in Primates Implicates the PRDM9 Gene in Meiotic Recombination

S. Myers et al.

10.1126/science.1182363

Prdm9 Controls Activation of Mammalian Recombination Hotspots

E. D. Parvanov et al.

A chromatin-modifying enzyme functions in the determination of recombination loci within the genome.
10.1126/science.1181495

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Primates clear an important hurdle in humanlike mastery of fire.

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Women's fingers are more sensitive, but that's just because they are smaller than men's are.

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RESEARCH ARTICLE: Regulation of Epidermal Growth Factor Receptor Trafficking by Lysine Deacetylase HDAC6

Y. Lissanu Deribe et al.

HDAC6 sets a brake that slows down the delivery of activated epidermal growth factor receptors to the degradative compartment.

RESEARCH ARTICLE: Tumor Suppression by PTEN Requires the Activation of the PKR-eIF2 α Phosphorylation Pathway

Z. Mounir et al.

PTEN provides a link between tumor suppression and the inhibition of protein synthesis independently of its regulation of PI3K signaling.

RESEARCH ARTICLE: Cbl Controls EGFR Fate by Regulating Early Endosome Fusion

G. D. Visser Smit et al.

The E3 ubiquitin ligase Cbl mediates the fusion of early endosomes necessary to target EGFR for lysosomal degradation.

PERSPECTIVE: Channeling Calcium—A Shared Mechanism for Exocytosis-Endocytosis Coupling

S. S. Vogel

Exocytotic insertion of calcium channels can couple exocytosis with endocytosis.

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S. Webb

Benjamin tenOever is an unconventional virologist who is working to make his discoveries clinically relevant.

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L. Laursen

The logistics can be intimidating, but a postdoc in the United States is rewarding.

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PERSPECTIVE: PPAR γ Activation—A Potential Treatment for Pulmonary Hypertension

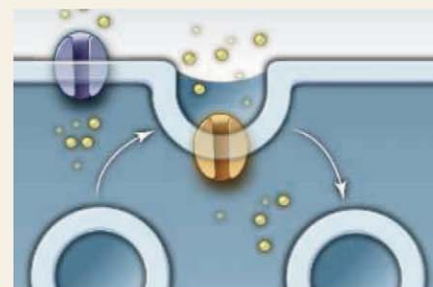
G. Hansmann and R. T. Zamanian

A new target is identified to thwart heart failure.

PERSPECTIVE: α_{2A} -Adrenergic Receptors in the Genetics, Pathogenesis, and Treatment of Type 2 Diabetes

S. B. Liggett

Results originating from rat genetics make a compelling case for the ADRA2A locus and type 2 diabetes in humans.



SCIENCE SIGNALING

Coupling exocytosis with endocytosis.

RESEARCH ARTICLE: Measurement and Clinical Monitoring of Human Lymphocyte Clonality by Massively Parallel V-D-J Pyrosequencing

S. D. Boyd et al.

Rapid sequencing of immune receptor loci can provide direct detection and tracking of immune diversity.

REPORT: Seasonal Influenza Vaccine Provides Priming for A/H1N1 Influenza Immunization

G. Del Giudice et al.

A vaccine currently used to protect against H1N1 is more effective when co-injected with an adjuvant and seasonal flu vaccine.

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Be-Deviled Cancer >>

Recently, a deadly transmissible cancer has emerged in Tasmanian devils, the largest existing marsupial carnivore. This disease, devil facial tumor disease (DFTD), leads to the growth of large facial tumors that frequently metastasize to internal organs. DFTD is thought to be transmitted by biting, and leads to death of affected animals within months, usually by obstructing the animals' ability to feed. Consequently, in the last 10 years Tasmanian devil numbers have dropped by about 60%. There are no genetic tests, vaccines, or treatments available for this disease, and without intervention, models predict that DFTD could cause extinction of Tasmanian devils in the wild within 50 years. Several lines of evidence suggest that DFTD is transmitted as a clonal allograft, whereby the cancer cells themselves are the agents of tumor transmission. **Murchison *et al.*** (p. 84) examined this hypothesis in detail by genotyping 25 tumor-host pairs from around Tasmania at 14 microsatellite loci and at a variable mitochondrial polymorphism. DFTD tumors were indeed found to be genetically distinct from their hosts and almost completely genetically identical to one another, supporting the idea of transmission by allograft.



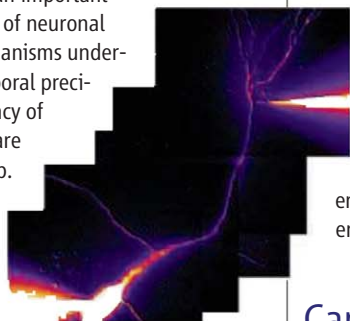
Lipid Rafts Come of Age

Living cells are surrounded by cellular membranes composed of lipids and proteins. Much attention has been paid to the biogenesis and sorting of membrane proteins. The dynamics and sorting of lipids have been much more difficult to study. **Lingwood and Simons** (p. 46) review the evidence for, and the role played by, so-called lipid rafts—laterally segregated regions within membranes enriched for particular lipids and proteins.

Dendrites Shape Interneuron Firing

Basket cells, a group of fast-spiking inhibitory interneurons, play an important part in the function of neuronal networks. The mechanisms underlying the high temporal precision and short latency of basket cell activity are unclear. **Hu *et al.*** (p. 52, published online 3 December) investigated dendrite functions in fast-spiking hippocampal basket cells

and found that action potentials are initiated in the axon and propagate back into the dendrites without activity dependence but with strongly reduced amplitude. This is very different from what has been observed previously in widely investigated pyramidal cell dendrites, probably due to the high potassium to sodium conductance



ratios in the dendrites of the interneurons. These dendritic mechanisms can explain the high-frequency firing and precise timing of basket cells seen in network activity in vivo.

Activating Stubborn Dopants

Many applications of semiconductor light-emitting diodes and lasers, such as reading optical disks, benefit from shorter wavelengths, but this requires materials with larger energy gaps between their valence and conduction bands. The electronic conductivity of these materials often has to be increased by doping with impurity atoms. However, in nitride materials, such as GaN and AlGaN, hole doping with acceptor atoms such as Mg is ineffective at room temperature. **Simon *et al.*** (p. 60) grew a gradient of AlGaN on the surface of GaN and found that the polarization of the layer could field-ionize the acceptor dopants efficiently at room temperature. The heterostructure was used successfully in a light-emitting diode that emits in the ultraviolet.

Carbon Nanotube Bridge for DNA Transport

The nanoporosity of carbon nanotubes has been exploited in the control of molecular transport—for example, in creating membranes. **Liu *et al.*** (p. 64) fabricated devices in which one single-walled carbon nanotube connects two fluid reservoirs. In some of these devices, apparently those

in which the nanotube is metallic, the ionic conductivity is anomalously higher than that expected from the bulk resistivity of the electrolyte. This high conductivity was exploited for the transport of single-stranded DNA, which was accompanied by large but transient increases in the ion current.

Metal in the Middle

Biphasic reaction mixtures allow the isolation of sensitive products, which can form in one solvent and then shift rapidly into another, protected from side reactions and interfering by-products. However, ensuring that catalysts remain in the proper phase can be challenging, and surfactants added to induce efficient mixing often prove difficult to separate from the product stream. **Crossley *et al.*** (p. 68; see the Perspective by **Cole-Hamilton**) tackle these issues by preparing easily recoverable amphiphilic nanoparticles that simultaneously stabilize aqueous-organic emulsions and catalyze organic reactions. The particles combine hydrophobic nanotubes with hydrophilic oxides, causing them to accumulate at water-hydrocarbon interfaces. Depositing palladium on specific portions of the particles' surfaces thus localizes the metal in one or both phases, facilitating hydrogenation of several compounds of interest in biofuel refining.

The Depths of the Changes

Over the course of the past glacial cycle, there have been two major types of rapid, large climate warming events: shorter-lived warm intervals lasting on the order of 1000 years and the

last glacial-interglacial transition. Although both involved dramatic changes in large-scale ocean circulation, the extent to which those changes were similar is unclear. **Roberts *et al.*** (p. 75) analyzed the neodymium isotopic composition of the Fe-Mn oxide coatings of planktonic foraminifera and reconstructed patterns of Atlantic Ocean circulation during Heinrich event 1, a rapid global climate fluctuation about 14,000 years ago involving the destruction of Northern Hemisphere ice shelves and the last deglaciation. While both the source of deep water and the whole-ocean overturning rate shifted rapidly and synchronously during the last deglacial transition, only upper ocean circulation strength was affected during Heinrich event 1.

Evolution in Action

Rates of evolution in gene and genome sequences have been estimated, but these estimates are subject to error because many of the steps of evolution over the ages are not directly measurable or are hidden under subsequent changes. **Ossowski *et al.*** (p. 92) now provide a more accurate measurement of how often spontaneous mutations arise in a nuclear genome. Mutations arising over 30 generations were compared by sequencing DNA from individual *Arabidopsis thaliana* plants. UV- and deamination-induced mutagenesis appeared to bias the type of mutations found.

We Are Stardust

Supernovae form as the result of stellar explosions and are classified according to the properties of their spectra. **Poznanski *et al.*** (p. 58, published online 5 November) present a peculiar supernova that is characterized by extremely fast temporal evolution and unusual spectroscopic features, such that it defies classification. SN2002bj appears to be a member of a new class of supernovae, possibly formed by a helium detonation on a white dwarf ejecting a small envelope of material.



Modifying Protein Modification

Alpha-dystroglycan (α -DG) is a cell-surface receptor that anchors the basal lamina to the sarcolemma by binding proteins containing laminin-G domains. This binding is essential for protecting muscle from contraction-induced injury, and defective binding is thought to cause a subclass of congenital muscular dystrophy (CMD) in humans. Mutations in six (putative) glycosyltransferase genes have been identified in patients with CMD, suggesting that glycosylation of α -DG may confer the ability to bind laminin. Despite extensive efforts for over 20 years, the actual laminin-binding moiety has remained unclear. Now, **Yoshida-Moriguchi *et al.*** (p. 88) have identified a phosphorylated *O*-mannosyl glycan on α -DG. This modification occurred in the Golgi via an unidentified kinase and was required for the maturation of α -DG into its laminin-binding form.

Flowery Regulator

Control of gene transcription is multilayered, depending on transcription factors, epigenetic mechanisms, and interactions with small RNA molecules. **Liu *et al.*** (p. 94, published online 3 December) have now found that for the *FLOWERING LOCUS C (FLC)* gene of the plant *Arabidopsis*, a backwards transcript of the gene conspires with 3' RNA-processing tools and histone demethylation to regulate the transcription of the protein-coding gene. The 3'-processing events require the antisense, not the sense, RNA transcript. It is then the sense transcript that, in the end, regulates onset of flowering.

Bacterial Compartmentalization

In diverse bacteria, reactions that involve toxic or volatile metabolites are carried out by enzymes inside proteinaceous microcompartments. **Tanaka *et al.*** (p. 81; see the Perspective by **Kang and Douglas**) now report high-resolution crystal structures for four homologous proteins that are constituents of the shell that sequesters the metabolism of ethanolamine in bacteria. While the structures have similar overall folds, they have distinctive structural features that provide insight into how they build the shell and participate in microcompartment function.

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Promoting Scientific Standards

THE SCIENTIFIC ENTERPRISE IS BUILT ON A FOUNDATION OF TRUST. AS KENNETH SHINE AND I emphasized 15 years ago in this journal, if science is to flourish and attain its appropriate role in aiding human progress, “It is incumbent upon all of us in the scientific community to help provide a research environment that, through its adherence to high ethical standards and creative productivity, will attract and retain individuals of outstanding intellect and character to one of society’s most important professions.”*

Journals such as *Science* occupy a special place in the maintenance of scientific standards. As an influential gatekeeper to the peer-reviewed literature across the natural and social sciences, what *Science* decides to publish helps to define scientific excellence for scientists. And with remarkable frequency, the broader media uses our selections to decide which scientific advances to convey to the public, adding to our profound sense of responsibility. For these reasons, the chief editors of the journals *Science*, *Nature*, and the *Proceedings of the National Academy of Sciences* have been working together to consider how to improve our procedures, so as to help make science as productive as possible in serving both scientists and the greater society. As a start, we have focused on two critical authorship issues.

First, to discourage “honorary authorships,” we agreed that before acceptance, each author will be required to identify his or her contribution to the research (see www.sciencemag.org/about/authors). *Science*’s policy is specifically designed to support the authorship requirements presented in *On Being a Scientist: Third Edition*, published by the U.S. National Academy of Sciences.† That report emphasizes the importance of an intellectual contribution for authorship and states that “Just providing the laboratory space for a project or furnishing a sample used in the research is not sufficient to be included as an author.”

Second, *Science* will require that the senior author for each laboratory or group confirm that he or she has personally reviewed the original data generated by that unit, ascertaining that the data selected for publication in specific figures and tables have been appropriately presented. Thus, for example, a researcher who prepares a digitally processed figure displaying an assortment of electrophoretic gel separations will need to present all of the original gel data to a specified senior author, who must certify that this has been done when the manuscript is returned for revision.

In this way, *Science* aims to identify a few senior authors who collectively take responsibility for all of the data presented in each published paper. Traditionally, a single individual has been asked to accept this responsibility. But the former requirement has become increasingly unrealistic, considering that a large fraction of publications now contain contributions from groups with very different expertise—and that half of the papers published in 2009 by *Science* had authors from more than one nation.

One issue not yet resolved is what scientific journals might do to encourage good mentoring practices by experienced scientists. Many universities now require that their young faculty members choose one or more mentors among the senior faculty. These mentors then use the wisdom and connections developed from their decades of experience to help the younger scientist in whatever ways are requested, including decisions that involve ethical standards. Being a good mentor resembles being a good parent: It involves a great deal of listening and help with problem solving and requires mutual respect and trust. Should the acknowledgments section of a publication specifically list any mentoring that made a major contribution to the research? Could a special “mentor search” function on PubMed (and on other literature compilation Web sites) then help to reward mentors?

Effective mentoring is critical to the future success of science, and as scientists remain active to more advanced ages, it provides a meaningful way to end a career. Scientists everywhere can and should do more to promote it.

—Bruce Alberts

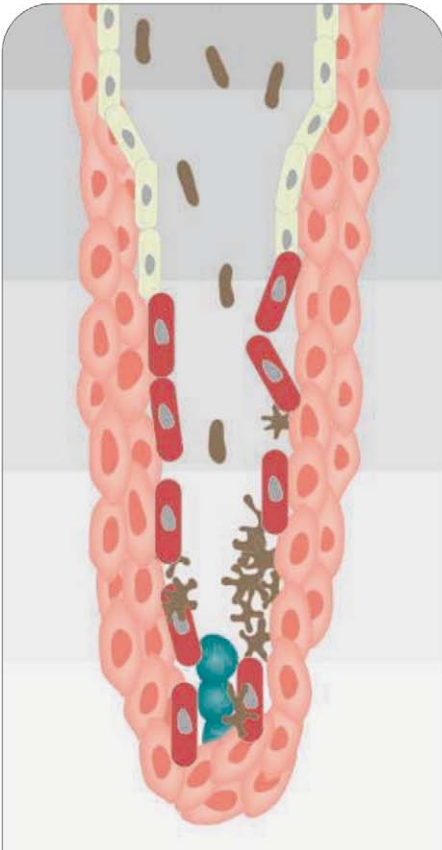
10.1126/science.1185983

*B. Alberts, K. Shine, *Science* **266**, 1660 (1994). †www.nap.edu/catalog/12192.html.



Bruce Alberts is Editor-in-Chief of *Science*.





BIOMEDICINE

Sealed with a Platelet

The fetal circulatory system has distinctive anatomical features because the fetus obtains oxygen through the placenta rather than through its lungs. Before birth, a blood vessel called the ductus arteriosus (DA) allows blood to bypass the nonfunctional fetal lungs by connecting the pulmonary artery, which supplies blood to the lungs, with the aorta, which supplies blood to the rest of the body. This vessel normally closes a day or two after birth, but in some newborns, it remains open and can lead to life-threatening complications. Studying newborn mice, Echtler *et al.* make the surprising observation that platelets—cells noted for their role in blood clotting—were recruited to the lumen of the DA within 20 minutes after birth of the mice; when platelet production or function was disrupted, the DA failed to close completely, leading to abnormal patterns of blood flow. The recruited platelets play a dual role in DA closure—by forming a physical plug that seals the lumen of the constricted DA and by altering the behavior of other cell types involved in blood vessel remodeling. — PAK

Nat. Med. 10.1038/nm.2060 (2009).

PHYSICS

Microwave Manipulation

Optical lattices populated by neutral atoms are a good candidate for storing quantum information. Normally, internal degrees of freedom such as the hyperfine state are used to create the basic information unit, the qubit. However, atoms also possess motional degrees of freedom; for example, the confinement of atoms in the lattice wells creates quantized vibrational states. These motional degrees of freedom are usually controlled by introducing time-dependent lattice potentials. Now, Förster *et al.* use microwave fields to effect transitions between vibrational levels of opposite hyperfine states of Cs atoms. Atoms in the two hyperfine states are loaded into two lattices spatially offset from each other. This arrangement enables transitions between different vibrational states, but the probability depends on the overlap of the (offset) wave functions. If the lattice is deep, transitions only happen between neighboring, slightly offset wells; if it is shallow, the offset can be increased and the atom becomes delocalized. Effective initialization into the lowest vibrational state is achieved, and Rabi oscillations between arbitrary vibrational states are demonstrated. This approach may lead to full control of quantum transport, likely a necessity for processing quantum information in this system. — JS

Phys. Rev. Lett. 103, 233001 (2009).

CELL BIOLOGY

In the Wild

Malaria is one of the most prevalent infectious diseases and kills around 900,000 people per year. It is caused by parasites of the genus *Plasmodium*, which are transmitted to humans by mosquitoes and enter red blood cells, causing fever and, if left untreated, death. Human pathogens of all kinds can develop resistance to the most effective drugs, such as artemisinin, so there is a constant need to identify new compounds. Animal models of malaria have proven problematic to establish, and most studies have used laboratory cultures of human blood cells to grow the parasites. While important insights into the life cycle and pathogenic action of *Plasmodium* have come from these in vitro studies, a recent study of clinically isolated samples of

Plasmodium in comparison to laboratory cultures revealed differences in gene expression profiles. Acharya *et al.* have analyzed the protein expression profiles of two species of *Plasmodium* that were isolated from the blood of patients; they identified about 100 proteins, some of which had not been found in laboratory cultures and could make promising drug or vaccine targets. — HP

Proteomics Clin. Appl. 3, 1314 (2009).

NEUROSCIENCE

The Next Top Model

Consumers may be familiar with high-end graphic processing components in video game consoles, such as the PlayStation3, or as a consequence of outfitting personal computers ordered online with NVIDIA graphics cards; these advances in hardware have also attracted the attention of procurement officials in the military services. In the academic realm, Pinto *et al.* have harnessed the power of clustered graphics processors to assess the relative performance of machine vision models of object recognition. The availability of massively parallel processing power at reasonable cost allowed them to explore, in 1 week versus 2 years, sizable regions of parameter space by varying the number of filters, the learning rate, and so forth. They generated a library of 7500 models that were trained on individually rendered objects during an unsupervised learning phase, and then screened on the basis of recognizing cars versus planes, which were presented in a range of orientations and on a variety of backgrounds. The top-ranked models were then evaluated broadly across other objects and on one of the toughest recognition tasks—photographs of human faces—and compared to a number of sophisticated algorithms, which yielded a small set of parameter values that were associated with high object recognition accuracy. — GJC

PLoS Comput. Biol. 5, e1000579 (2009).





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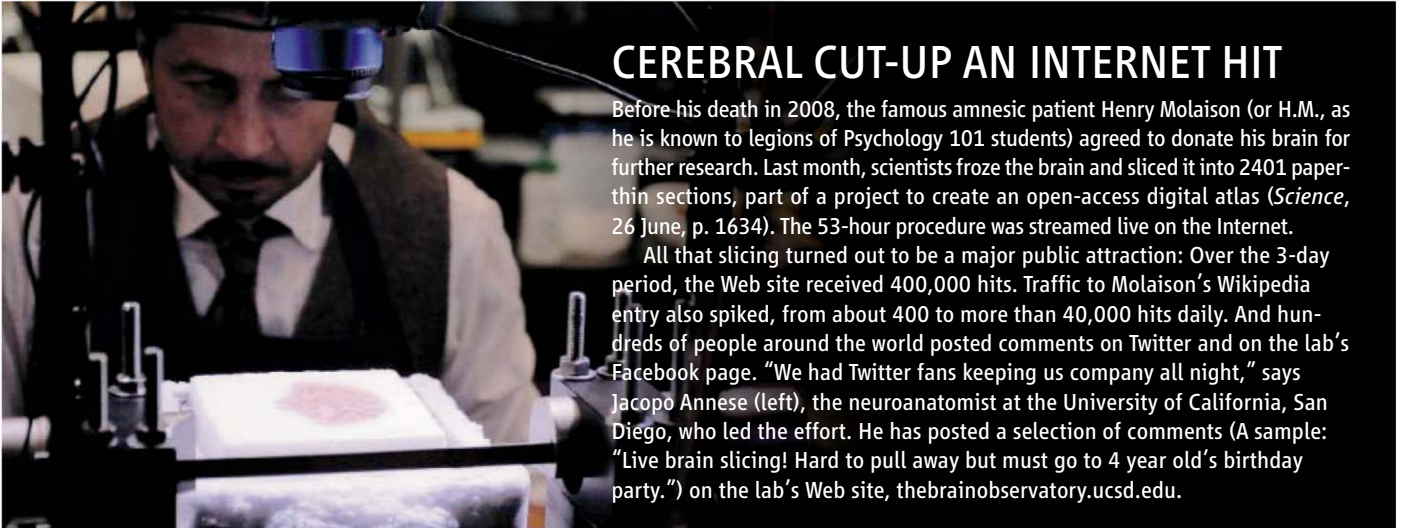
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CEREBRAL CUT-UP AN INTERNET HIT

Before his death in 2008, the famous amnesic patient Henry Molaison (or H.M., as he is known to legions of Psychology 101 students) agreed to donate his brain for further research. Last month, scientists froze the brain and sliced it into 2401 paper-thin sections, part of a project to create an open-access digital atlas (*Science*, 26 June, p. 1634). The 53-hour procedure was streamed live on the Internet.

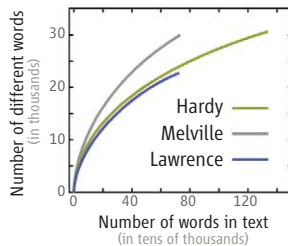
All that slicing turned out to be a major public attraction: Over the 3-day period, the Web site received 400,000 hits. Traffic to Molaison's Wikipedia entry also spiked, from about 400 to more than 40,000 hits daily. And hundreds of people around the world posted comments on Twitter and on the lab's Facebook page. "We had Twitter fans keeping us company all night," says Jacopo Annese (left), the neuroanatomist at the University of California, San Diego, who led the effort. He has posted a selection of comments (A sample: "Live brain slicing! Hard to pull away but must go to 4 year old's birthday party.") on the lab's Web site, thebrainobservatory.ucsd.edu.

Name That Author

If you're a casual reader who has trouble telling your Updike from your elbow, a team of physicists may be able to help you. Sebastian Bernhardtsson and colleagues at Umeå University in Sweden say they can distinguish one author from another by analyzing their writings statistically. They tracked how the number of different words in a sample of text grows with the total number of words in the sample for three authors: Herman Melville, D. H. Lawrence, and Thomas Hardy. Each writer has his own distinctive curve describing that increase, the physicists recently reported in the *New Journal of Physics*.

In a short sample, the number of different words increases almost as fast as the total does; it increases more gradually as the sample becomes longer. So the curves start out steep and eventually level out. Melville, who uses the biggest vocabulary, has the steepest curve. Hardy adds new words at a slower rate, followed by Lawrence.

If the method works, "that would be interesting because it's such a simple statistic," says Daniel Rockmore, a mathematician at Dartmouth College. But he says the researchers would have to compare many more authors to prove it. R. Harald Baayen, a quantitative linguist at the University of Alberta in Edmonton, Canada, says others have been working on such statistical methods for decades and that the physicists' method may be too simple. Variations among one author's books, he notes, often exceed the variations between authors.



Myopia Out of Control

Americans are getting ever more nearsighted, according to scientists at the National Eye Institute in Bethesda, Maryland. In the early 1970s, about 25% of the population qualified as myopic. In the early 2000s, that proportion had leapt to almost 42%, says a team led by epidemiologist Susan Vitale.

The report, in the *Archives of Ophthalmology*, created a buzz last week about the dangers of computers and texting. Similar increases have been happening around the world.

Oddly, the condition is highly heritable yet

malleable by the environment. But what environment? "The suspicion has always been centered around 'near work,'" says vision scientist Donald Mutti of Ohio State University in Columbus. But recent studies of schoolchildren in both the United States and Singapore have failed to show an association between near work and an increase in myopia. Mutti says research instead is increasingly pointing to lack of outdoor exposure as the culprit. "We're kind of a dim indoors people nowadays," he observes. "If you ask me, I would say modern society is missing the protective effect of being outdoors"—although whether it's the light or the distance that does it is still not known.

Mammoths' Last Stand

Most of North America's large mammals are thought to have gone extinct some 13,000 to 15,000 years ago. But a new study of ancient DNA suggests that woolly mammoths and horses hung on until 10,000 years ago or later.

A multinational team headed by geneticist Eske Willerslev of the University of Copenhagen reached that conclusion by analyzing soil samples for ancient DNA from animals' urine and feces.

The team picked a site on the banks of the Yukon River in central Alaska, a likely mammoth stomping ground where the permafrost has never melted and the stratigraphy is well dated. They took core samples from seven permafrost layers ranging from about 12,000 to about 7500 years old. The scientists reported last week in the *Proceedings of the National Academy of Sciences* that they found mitochondrial DNA from mammoth, bison, moose, horse, and snowshoe hare. Mammoth and horse DNA was dated to between 10,500 and 7600 years ago—postdating the most recent fossils by at least 3000 years. The



results show that some survived in the interior for several thousand years after the arrival of their human predators, the researchers say.

Paul Koch, a paleoecologist at the University of California, Santa Cruz, says that obtaining "sedimentary" ancient DNA is relatively new and that the results put "another nail in the coffin" of the idea that large North American mammals went extinct suddenly.



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U.S. SPACE POLICY

Obama Backs New Launcher And Bigger NASA Budget

President Barack Obama plans to ask Congress to cancel work on a new rocket and instead fund a heavy-lift launcher to take humans to the moon, asteroids, and the moons of Mars. The president outlined the new direction for the U.S. human space flight program on 16 December at a meeting in the White House with NASA Administrator Charles Bolden, according to officials familiar with the discussion. NASA would see its 2011 budget grow by \$1 billion both to get the new launcher on track and to bolster the agency's fleet of robotic Earth-monitoring spacecraft.

The current NASA plan for human exploration is built around the \$3.5 billion Constellation program, which was intended to provide a way to get humans to the space station and beyond after the space shuttle is retired this year. But its initial launcher, Ares 1, has faced a string of cost and technical problems, and an outside panel chaired by retired aerospace executive Norman Augustine was skeptical of that effort (*Science*, 25 September, p. 1606). Although NASA has done a good job confronting the rocket's engineering challenges, says Augustine, "the schedule has slipped so badly it doesn't fit into the future program well." But some lawmakers, such as Senator Richard Shelby (R-AL), are sure to fight any changes to the program.

According to knowledgeable sources, the White House has decided that scarce NASA funds would be better spent on a simpler heavy-lift vehicle that could be ready to fly as early as 2018. Meanwhile, Europe, Japan, and Canada would be asked to work on a lunar lander and modules for a moon base, a contribution that would save the United States several billion dollars. And commercial companies would take over the job of getting supplies and possibly humans to the international space station.

"The decision is not going to make anyone gasp," said one source in the White House, which hopes to ease congressional concerns about the impact of the new plan on existing aerospace jobs by transitioning workers from Ares 1 to the heavy-lift vehicle



Change in direction. President Obama has told NASA Administrator Charles Bolden (*inset*) that he wants to replace Ares 1 (*above*) with a heavy-lift rocket.



project. But Shelby and some of his colleagues fear that an Ares 1 cancellation will lead to mass layoffs in their states. Indeed, Shelby inserted language into the 2010 NASA spending bill that requires the agency to gain congressional approval before changing the existing rocket program.

Last month, Shelby also wrote to NASA's inspector general asking his office to investigate alleged conflicts of interest on the Augustine panel. The legislator said that several panel members were registered lobbyists who took "direct advantage of their temporary roles on the Commission to further their personal business." None of the panel members was actually a registered lobbyist, although Augustine says, "I'd be surprised" if lobbyists had not provided the panel with their input.

The form that the heavy-lift launcher would take has yet to be decided. But rather than pointing the rocket to the moon, as U.S.

President George W. Bush proposed in 2004, this White House is more intrigued by human missions to asteroids, Phobos, and Deimos as a precursor to landing humans on the Red Planet. That option was given particular prominence by Augustine panel members when they testified this fall before congressional committees. To prepare for human visits, NASA may order additional robotic missions to the martian moons and asteroids.

Before making his decision, Obama reviewed several options presented to him by NASA, the Office of Management and Budget, and the Office of Science and Technology Policy. The choices included keeping NASA's budget flat and delaying a new launcher, giving it an additional \$1 billion to build a heavy-lift launcher, ramping up NASA's annual budget by \$3 billion for an aggressive program, or reducing NASA's budget and abandoning space flight. The

president's decision to go with the second option is a major departure from his 2010 budget plan, which called for NASA to receive a 5% increase in 2010 followed by level funding through 2014.

The Augustine panel concluded that NASA needed a \$3 billion annual increase to move ahead with a robust space-flight program. Last month,

Congress belatedly completed action on NASA's 2010 budget, boosting it by \$1 billion, to \$18.7 billion. An additional \$1 billion in 2011, combined with support from other countries, would put the agency close to the panel's suggested level. "There are a lot of different ways to reach that level, including help from abroad and increasing NASA's efficiency," Augustine added.

It's not clear when the new policy will be formally announced. One White House source said it was imminent, while another hinted that it would wait until Obama's State of the Union address in late January. Another possibility is a 1 February release as part of the president's 2011 budget request to Congress. Given the White House's preoccupation with health care and climate change, however, NASA officials and their industry backers see the new policy as welcome proof that Obama also cares about space flight.

—ANDREW LAWLER

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CLIMATE CHANGE

Senate Looms as Bigger Hurdle After Copenhagen

President Barack Obama knew the clock was ticking on reaching a climate-change agreement when he barged into a meeting with the leaders of China, Brazil, India, and South Africa on the last day of the deadlocked U.N. convention in Copenhagen. And while Obama called the 18 December deal he brokered an “unprecedented breakthrough” in getting developing countries to agree for the first time to voluntary reductions in greenhouse-gas emissions, he knew that it was missing major components that supporters had sought, ranging from mandatory mitigation targets to deforestation. The so-called Copenhagen Accord (see chart) didn’t even contain a timetable for deciding when to decide.

But as hard as it was to negotiate a deal in Denmark, Obama faces an even tougher time in Washington trying to convince the U.S. Senate to pass cap-and-trade legislation. And he’s up against another ticking clock: the November 2010 elections that will decide whether his party retains control of Congress. “The closer you get to the election around here, the less policy is going on and the more politics,” says Senator Lisa Murkowski (R-AK). Even so, Murkowski predicts that the Senate will need “months, ... not weeks” to debate the complex issue.

One major obstacle is the Senate’s own rules. A supermajority is needed to pass nearly any controversial legislation, because opponents can launch a filibuster that requires 60 votes to bring to an end. Supporters can count on only about half that number, and getting the rest will require deals with some combination

of coal-state Democrats, moderate Republicans like Murkowski, and liberals who want a stronger bill. And that means balancing concerns about trade, energy, and the economic effects of the bill. “It’s going to be a tough environment to find 60 votes,” says Thomas Gibson of the American Iron and Steel Institute.

For advocates of emissions reductions, the less-than-satisfying outcome of the Copenhagen summit shows how difficult it will be to reach any agreement. In June, the House of Representatives narrowly passed landmark emissions reductions that would bring U.S. emissions 20% below 2005 levels by 2020. And such legislation hasn’t fared well in the Senate. In 2003, cap-and-trade legislation went down by a margin of 43 votes to 55. Two years later, it secured only 38 votes. In 2008, a procedural vote on a bill that contained more favorable language for nuclear power fell 12 votes short of the 60 required to bring it to a vote—although six senators said they would have voted for the bill had they been present.

This time around, legislation introduced in October by Senators John Kerry (D-MA) and Barbara Boxer (D-CA) and approved the following month by the Senate Environment



Dealmakers. President Obama engaged fellow world leaders on the last day of the Copenhagen meeting.

and Public Works Committee has already attracted plenty of ill will. Republicans on the committee boycotted the vote, and Boxer, the chair, bucked precedent by proceeding without them. And hers is the only one of six committees with jurisdiction to have passed the legislation.

Democrats from states where coal is king will be central to any successful deal. Last month, 92-year-old Senator Robert Byrd (D-WV), their unofficial leader, shocked the coal industry when he declared that “West Virginians can choose to anticipate change and adapt to it, or resist and be overrun by it.” He also said he plans to “stay at the table” to make sure his state is “part of any solution,” increasing the chances that the Senate bill would provide more money for clean-coal projects ▶

TWO WEEKS IN DENMARK

ISSUE	WHAT HAPPENED IN COPENHAGEN	WHAT REMAINS UNRESOLVED
 Reducing Emissions	Agreed to hold temperature rise to 2°C by 2100; each country will report this month on plans for voluntary cuts between 2010 and 2020.	Agreeing on an emissions goal for 2050 and legally binding cuts by both developing and developed nations.
 Verification	Agreed to have developed nations report their emissions cuts, subject to international verification. Developing countries will draw upon “international consultations” in reporting their performance.	Agreeing on a mandatory process to check numbers from developing nations, with consequences if the numbers are wrong.
 Financing	Developed nations agreed to provide roughly \$30 billion between 2010 and 2012 for mitigation and adaptation, rising to \$100 billion a year by 2020.	Agreeing on who will pay what, when, and how the money will be distributed.
 Deforestation	The financing would include \$3.5 billion for developed nations to pay tropical countries to slow the destruction of forests.	Agreeing on the scale—national or regional—to be used in counting efforts to avoid deforestation.

CREDITS (TOP TO BOTTOM): PETE SOUZA/WHITE HOUSE/SIPA PRESS/NEWS.COM; (SOURCE) U.N. FRAMEWORK CONVENTION ON CLIMATE CHANGE AND SCIENCE

and assistance to industry than the House version. Senator Tom Carper (D-DE), who led a task force on coal for Boxer earlier this year, says he can already see momentum building. "If the House members from a coal state voted for the House bill, it's going to be hard for some senators to ignore what their House members have done," says Carper.

Bridging the gap between coal-state lawmakers and environmentalists is not the only difficult task facing the White House. Getting a handful of Republicans to support the bill is another crucial one. One key step in that direction is an outline of cap-and-trade legislation released last month by senators Kerry, Joe Lieberman (ID-CT), and Lindsay Graham (R-SC). Last month, Graham said the legislation, if passed, would "create jobs, protect our national security interests, and improve our environment."

Winning Graham's vote might require more support for the nuclear industry than is in the House legislation, including advanced research, regulatory changes, and some type of financial incentives for new nuclear power plants. Graham's role is the "greatest indication that you can reach a 60-vote margin," says Lou Leonard of the World Wildlife Fund in Washington, D.C. On the other hand, such concessions could cause a mutiny among staunch liberals like Senator Bernie Sanders (I-VT), who says he would consider voting against the bill depending on "how weak it is."

One unknown may be fallout from the leaked climate science e-mails (*Science*, 4 December, p. 1329), which had virtually no effect on negotiations in Copenhagen. Senator

Online

sciencemag.org

For interviews and analyses, and to learn where individual senators stand on climate bills, go to <http://scienceinsider.org>.

James Inhofe (R-OK), an outspoken climate skeptic, believes the scandal spells doom for climate legislation that he's already labeled "dead on arrival." But two other Republicans—senators Murkowski and Lamar Alexander (R-TN)—told *Science* that the e-mails have not shaken

their confidence in the science behind global warming. Indeed, Boxer told reporters at a press conference this week that the new push by foes to challenge the fundamental facts of global warming will backfire because it will put "new attention on the science—and that's good."

It's not clear how the watered-down Copenhagen pact might affect the Senate vote. Before the summit, Boxer said that a political agreement there would "help us

RESEARCH MANAGEMENT

Protests by Staff, Advisers Rattle Australian Synchrotron

MELBOURNE, AUSTRALIA—

Amid media fanfare and the smiles of politicians, the Australian Synchrotron (AS) here unveiled a new tunnel in April that will eventually house a medical beamline. Leaders said it would push the boundaries of synchrotron science, allowing clinicians to image a single cancer cell in a woman's breast or plaques in the artery wall of a beating heart. In June, the \$330 million synchrotron won funding for a new science center and a hotel complex. In September, it hosted a major conference. Four months later,

the entire facility was in turmoil. Director Robert Lamb had been fired; a majority of the international scientific advisory committee (SAC) had resigned; and staff began a work-to-rule slowdown, demanding that the board chair, attorney Catherine Walter, be removed.

What went wrong? The crisis erupted shortly after the AS board removed Lamb from his job on 30 October. Lamb and board members have refused to discuss details. But many observers say that a gaping rift developed between the board of directors on one hand and AS staff and SAC on the other over the slow pace of developing long-term plans.

The AS is one of 17 facilities of its kind in the world. It accelerates a thin beam of elec-



Happier days. Chemist Robert Lamb, former director of the Australian Synchrotron, before his dismissal.

trons around a ring the diameter of a football field, emitting intense x-rays that are filtered through beamlines for customized applications. This third-generation AS was completed in 2007; it has been a "smashing success," says Jeff Corbett, a synchrotron scientist from the SLAC National Accelerator Laboratory in Menlo Park, California, and an AS adviser. Last year, the AS ranked second in the world in reliability, with a beam availability of 98%. Nine beamlines have been completed or are under way, but there are 29 vacancies and no new lines since 2007.

In a recent interview, Lamb, chair of chemistry at the University of Melbourne, said his relations with the board of directors

and its chair were cordial—right up to the day he was fired. Lamb says that Walter and Rod Hill, board deputy chair and pro vice-chancellor for industry engagement and commercialisation at Melbourne's Monash University, met in Lamb's office: "I thought, amongst other things, I was going to get a pat on the back. Instead, they told me I was gone. The whole thing lasted about 15 minutes." He claims that he has received "no official explanation as to why."

Initially, the board said little. But as concerns mounted among the staff and SAC, Walter, Hill,

and a third board member, Sean Gallagher—director of the University of Sydney's United States Studies Centre—met and spoke with the staff on 9 December. According to a text obtained by *Science*, Walter said that "legal and confidentiality constraints" prevented discussion of Lamb's firing. She spoke of the board's "deep concern about progress with the science and investment cases, which are crucial to obtaining funding for further development and expansion after 2012." An audit by the Victorian government last year praised AS science but raised a red flag over the lack of long-term science and business plans, according to one insider. In an e-mail

CREDIT: COURTESY OF ROBERT LAMB

move a climate-change bill forward.” But a central issue left unresolved in Denmark is China’s refusal to agree to international verification of its reports on emissions reductions. Obama won the support of the China delegation for compromise language that embraces “international consultations.” But without a firmer Chinese commitment, it may be hard to convince leery lawmakers that China and other major emitters from developing nations are willing to carry their share of the burden.

“There’s nothing [in the text] to keep these countries from accelerating their emissions,” says an Inhofe spokesperson, who says the talks “were a complete disaster” in terms of the pending legislation. The spokesperson also criticized the U.S. decision, announced at the talks, to participate in a \$100-billion-a-year fund, starting in 2020, for helping the developing world cope with global warming:

response, Walter said that the review “highlighted a priority that was also a concern of the board,” but she denied that it prompted the “scapegoating” of Lamb.

One of the board’s critics, Frank Larkins, chief scientist for energy of the Victoria State Government and SAC chair until his resignation on 9 December, says a “clash of cultures” separated the board from the scientific staff and SAC. “Our consistent problem has been the silence in response to our [SAC] recommendations” to the board to get moving on long-term science and business plans, says Larkins. He has urged Victoria to intervene and replace the chair of the board.

Michael Grunze, a professor of applied physical chemistry at the University of Heidelberg in Germany, another SAC member who resigned on 9 December, also blasted the board in a resignation letter. The SAC’s advice, Grunze wrote, “has been either consistently ignored or not acted upon in a timely manner by the leadership of the AS Board.”

Some critics of the board say that AS management started long-term planning at the beginning of the year but were held back. They accuse the board of micromanagement and choking operations with paperwork. “The board is much more involved in the administration than is necessary or good,” said Janet Smith, a protein crystallographer at the University of Michi-

“It probably made things worse, since the American people aren’t going to be happy seeing their tax dollars going abroad to support a global warming fund.”

Obama will also have to decide how much political capital to spend on an issue that is losing support among Americans, according to opinion polls, when one they care a great deal about—unemployment—is at 10% and could get worse. Some critics note that Obama didn’t say in Copenhagen that climate change would be a top priority in 2010. But Daniel Weiss of the liberal Center for American Progress in Washington, D.C., says that Obama’s meetings with world leaders at the conference and with U.S. politicians at home show he is fully engaged. “He’s succeeded in Copenhagen at pushing the process forward. Now he has to succeed at getting the Senate to act,” says Weiss.

—ELI KINTISCH

gan Medical School in Ann Arbor and a continuing SAC member.

Walter contests the view that the board did not act on SAC’s recommendations. In an e-mail she wrote, “All recommendations from SAC were passed on to the AS management.” And Gallagher said in a telephone interview that paperwork is an inevitable result of the organization’s complex structure. Stakeholders include five state governments, the federal government, New Zealand, 32 universities, and 42 other organizations.

Several SAC members have spoken about their concern for the synchrotron’s future. Soichi Wakatsuki, a

continuing member and director of the Photon Factory in Tsukuba, Japan, wrote in a 12 December letter to the board that being a member of SAC had been “the most distressing period in my career,” and that the status of AS “as a world class synchrotron” is now “in danger.” Ted Baker, a University of Auckland professor of structural biology and the new SAC chair, also warned the board, “if the level of disclosure and trust are not improved, radically, and fast, the AS will lose key staff and its use base will evaporate.”

The staff slowdown—which limits work to a standard 9-to-5 day—is ongoing at press time. The State Government of Victoria, which has the largest stake in the project, so far has declined to intervene.

—ELIZABETH FINKEL

“I thought ... I was going to get a pat on the back. Instead, they told me I was gone.”

—ROBERT LAMB,
AUSTRALIAN
SYNCHROTRON

ScienceNOW.org

From *Science’s* Online Daily News Site

Hand Size—Not Sex— Determines Sense of Touch

Sometimes a difference between the sexes is not based on sex at all.

Women have a finer sense of touch than men do, but a new study shows that this is simply because their fingertips tend to be smaller. <http://bit.ly/handsize>



Chimps Keep Their Cool With Fire

When primatologist Jill Pruetz found herself threatened by wildfires in the savannas of Fongoli, Senegal, in 2006 she had two options: stay with the chimpanzees she was studying, or run. She chose the chimps. The primates were calm, and—with her in tow—they carefully made their way around the blaze. The chimps’ actions, Pruetz would later report, set them apart from other non-human animals—and they may reveal the evolutionary origins of how we came to master fire. <http://bit.ly/chimpfire>

Seasick? Try Controlling Your Breathing

If you get seasick easily, you may prepare for boat rides with pressure-point bracelets, ginger, or a prescription skin patch. Now there’s one more remedy: timing your breathing to counteract the nauseating motion. The technique presumably works because it helps control gravity sensors in the abdomen—a lesser-known input to our fine-tuned balance system. <http://bit.ly/breathingtiming>

Bacteria Can Transform Minerals Electrically

Got a messy cleanup problem that requires a molecule-by-molecule fix? Instead of nanotech, how about deploying an array of ready-made, versatile bacteria? Scientists studying a genus of the rock-dwelling bacteria called *Shewanella* have found out how the organisms can transform minerals by zapping them with tiny electrical currents. The discovery could lead to new types of fuel cells to generate electricity, to better environmental-cleanup techniques, and possibly even to a new generation of organically made materials. <http://bit.ly/Shewanella>

Read the full postings, comments, and more on scienconow.sciencemag.org.

RESEARCH FUNDING

U.K. Physicists Cry Foul At Major Budget Cuts

It's not been a festive time for many U.K. physicists following the mid-December announcement of a 5-year funding plan for the Science and Technology Facilities Council (STFC), the British body responsible for particle physics, astronomy, nuclear physics, and space science. Administrative changes and the ravages of the economy have left the council with a gaping hole in its finances, so researchers braced for cuts and even offered advice on how to make them. Although the STFC tried to spread the losses fairly, nuclear physics was pared to the bone, with just £30 million for the next 5 years. This amounts to a cut of 29% over that period, according to nuclear physicist William Gelletly of the University of Surrey. "If the U.K. is serious about nuclear new build and maintaining high standards in nuclear medicine, ... then it should take a very hard look at how much it should be spending on nuclear physics," he says.

Although astronomy was cut less severely, by 10%, the U.K. government will

withdraw support from a number of projects, including the Pierre Auger Cosmic Ray Observatory, the Liverpool Telescope, and the U.K. Infra-Red Telescope. And in 2012 it will withdraw from the Gemini Telescopes, the James Clerk Maxwell Telescope, and the Isaac Newton Group of Telescopes. After that, U.K. astronomers will no longer have access to a major observatory in the Northern Hemisphere, their own sky. "We are now seriously concerned at the effect the loss of so many smaller projects will have on the health and morale of physics groups in British universities," says Andrew Fabian, president of the Royal Astronomical Society.

The STFC's problems date back to 2007, when it was created from the merger of two existing research councils, and nuclear physics moved into it from a third (*Science*,

Darkening skies. STFC threatened to cut U.K. funding to the Gemini telescopes in 2007. Money will now be withdrawn in 2012.



STFC	Science field	Total 5-year budget	Cut
	Astronomy	£267 million	10%
	Nuclear physics	£30 million	29%
	Particle physics	£690 million	4%
	Space science	£639 million	6%

21 December 2007, p. 1851). Both of the merged councils had overcommitted themselves to costly international projects, and STFC soon found a £40 million hole in its budget. The situation has since gotten worse as the weakening pound makes subscriptions to international facilities more expensive. And with the poor economy, the STFC expects to get no budget increase in the

SCIENTIFIC PUBLISHING

Errors in Chemistry Claims Cast Doubt on Reactome Paper

A newly developed research tool called a reactome array, which has attracted widespread interest from biologists, has come under intense fire from scientists who say the description of the device in the 9 October issue of *Science* (p. 252) includes "impossible" chemical reactions and makes little sense. The publication drew immediate attention because the array promises to establish the functions of a myriad of enzymes and probe the metabolism of bacteria and other kinds of cells.

Last week, *Science* acknowledged the furor, publishing online an "Editorial Expression of Concern" in which the journal's editor-in-chief, Bruce Alberts, notes that "serious questions have been raised about the methods and data presented." "It was just so obvious the chemistry was flawed," says biochemist Laura Kiessling of the University of Wisconsin, Madison, editor-in-chief of *ACS Chemical Biology*.

While admitting to serious errors in describing the methods used to create the reactome array—which required the synthesis

of thousands of compounds representing metabolites and other enzyme substrates, linking them to a fluorescent dye, and fixing them on a glass slide—the study's corresponding authors stand behind the device. "We're confident in the results and technology. Many researchers have used the array without problems," says Manuel Ferrer of the Spanish National Research Council's (CSIC's) Institute of Catalysis in Madrid. According to Ferrer, at the request of *Science*, CSIC will conduct an investigation of his lab, and related inquiries will be held at collaborators' labs in Germany and the United Kingdom. He says that Nobel laureate Richard Roberts of New England Biolabs and other researchers have also agreed to conduct blinded tests to verify that the reactome array works. Roberts confirmed that fact, noting that he recently visited the Madrid lab and came away "impressed" after initially thinking the reactome array "was too good to be true."

In private chats and online postings, chemists began expressing skepticism about

the reactome array as soon as the article describing it was published, noting several significant errors in an initial figure. Some also questioned how a relatively unknown group could have synthesized so many complex compounds. The dismay grew when supplementary online material providing further information on the synthesized compounds wasn't available as soon as promised. "We failed to put it in on time. The data is quite voluminous," says co-corresponding author Peter Golyshin of Bangor University in Wales, a microbiologist whose team provided bacterial samples analyzed by Ferrer's lab.

Science is also coming under fire. "It was stunning no reviewer caught [the errors]," says Kiessling. Ferrer says the paper's peer reviewers did not raise major questions about the chemical synthesis methods described; the journal's executive editor, Monica Bradford, acknowledged that none of the paper's primary reviewers was a synthetic organic chemist. "We do not have evidence of fraud or fabrication. We do have

CREDIT: (PHOTO) GEMINI OBSERVATORY PUBLIC INFORMATION & OUTREACH

United Kingdom's next 3-yearly comprehensive spending review.

Stung by criticism that its 2007 cuts involved no consultation, the STFC carried out an extensive prioritization exercise with the research community before announcing its latest funding plan. For 5 years from fiscal year 2011–12, the STFC will save £115 million by withdrawing from 24 major national and international projects and squeezing the budgets of 38 others. It will also require a 10% cut in grants to university researchers involved in those projects and a 25% cut in new postgraduate studentships and postdoctoral fellowships.

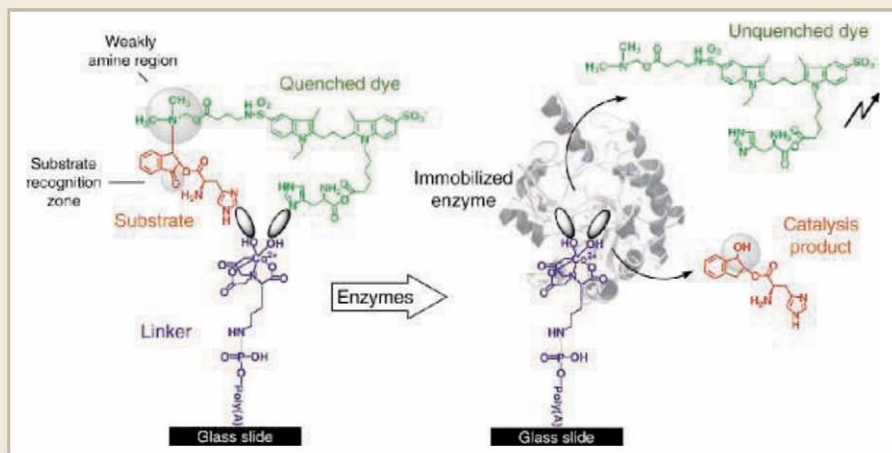
Particle physics, now mostly focused on exploiting CERN's new Large Hadron Collider, got the largest slice of the 5-year pie, £690 million, and had the smallest cut, 4%. In contrast, nuclear physicists, as STFC's newcomers, feel they've been sidelined by the much bigger communities already in place. "The cuts in nuclear physics will be devastating for the field. The number of academic nuclear physics researchers will drop by at least a factor of 2 from [currently around] 55," says Paul Nolan of the University of Liverpool. "At this level, they will not be able to make an international impact."

The STFC says that the decisions were

made by peer-review panels, reflecting the quality of the science. But some nuclear physicists argue that it is hard for such panels to be fair when some fields are poorly represented. "When the going gets rough, most people will tend to mark up the things they know and love. ... So it is no surprise that the big battalions, who make up 90% of the membership of these committees, fared better," says Gelletly. Others point out that at the end of the 5 years, the STFC has chosen to give its in-house facilities—the DIAMOND x-ray source and the ISIS neutron source—a £25 million increase, while cutting funds to external centers. "There is a major conflict of interest here," says Nolan.

Researchers are now talking about whether the STFC should be reorganized, separating facility funding from research grants, and whether it should oversee studentships and fellowships. "These are generally considered essential for the health of the science, and cuts make little sense," says Robert Cywinski of the University of Huddersfield. "Perhaps it is time that the whole concept of funding research studentships across all research councils should be reviewed and possibly taken out of the hands of the individual councils."

—DANIEL CLERY



Figured out. After spotting errors in this figure and being unsatisfied by supplementary online material, chemists challenged whether the so-called reactome array could work as claimed.

concerns about the inconsistencies and have asked the authors' institutions to try to sort all of this out by examining the original data and lab notes," she says.

Ferrer says he takes responsibility for all the mistakes and apologizes: "I understand the disappointment of *Science* and *Science's* readers." Yet some chemists, including those who have sought clarifications from the authors, remain unconvinced

by supplementary data that has since been posted and the explanations offered so far. Many colleagues "think it must be fraud. I'm trying to keep an open mind," says chemical biologist Ben Davis of the University of Oxford in the United Kingdom, who wrote a skeptical review of the reactome array article on the Faculty of 1000 Web site. "But clearly there are a lot of unexplained elements."

—JOHN TRAVIS

ScienceInsider

From the Science Policy Blog



Merck has hired the former director of the U.S. Centers for Disease Control and Prevention, **Julie Gerberding**, as its head of vaccine development. The company says the infectious-disease expert will expand the company's vaccine offerings internationally. <http://bit.ly/4R7nOL>

Henrik Thomsen, a Danish clinician who alerted patients and regulators to **potential risks of Omniscan, a drug used to improve MRI scans**, is facing a libel suit. Manufacturer GE Healthcare says a 2007 presentation by Thomsen was defamatory. <http://bit.ly/4Mb1qp>

The National Football League has changed its stance on the issue of **concussions and long-term brain-damage risk**. Doctors and experts working for the league had dismissed the link before, but now the NFL is exploring a partnership with scientists at Boston University who have been among its harshest critics. The partnership could include funding for the school's research. <http://bit.ly/6cps5f>

A new report details alleged errors associated with the "Propatria study," a **research trial into the use of "probiotic" therapies** in which live microorganisms are administered to patients to treat disease. The Dutch Health Care Inspectorate found that scientific rules were not followed in the study, in which 24 patients receiving the treatment died. By comparison, there were nine deaths among those receiving the placebo. <http://bit.ly/4NbieG>

In an interview with *ScienceInsider*, retiring science committee chair **Representative Bart Gordon (D-TN)** said that science education was among his top priorities for his last year in office. He'll be tackling that as part of a reauthorization of the America COMPETES Act. "One of the things we've been doing is inventorying all the current STEM education programs, and we're finding tens of millions of additional dollars being spent on programs that nobody knew about," said the lawmaker. <http://bit.ly/5Bz1sw>

For more science news and analysis, visit <http://scienceinsider.org>

HIGHER EDUCATION

Recession Hits Some Sciences Hard At Florida State University

Philip Froelich, 63, is the tenured Francis Eppes Professor of Oceanography at Florida State University (FSU) in Tallahassee. He won't be much longer. Despite a distinguished 31-year career as a researcher and administrator, he will be laid off next May. And despite a positive external evaluation within the past year, his department—much diminished by layoffs—will be no more, folded along with the geological sciences department into a new department dominated by meteorology. “Why would you cut Flip Froelich? It doesn't make any sense,” says geologist Michael Perfit of the University of Florida (UF), Gainesville.

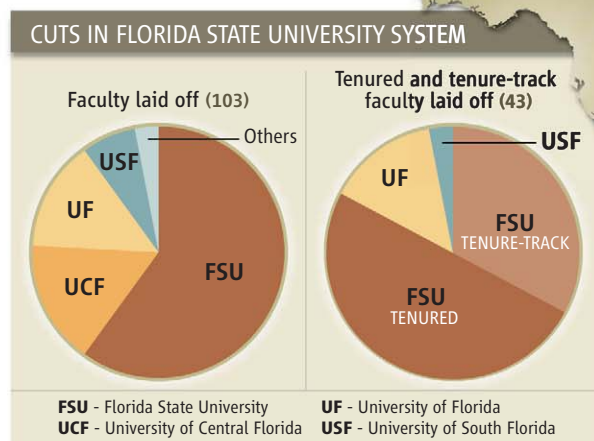
It's all about money, of course. When the cash-strapped Florida state legislature recently slashed funds for higher education for the third straight year, big across-the-board cuts spilled down through individual state university budgets. But at FSU—one of the “big four” Florida state schools—the fiscal crisis has turned into a ravaging torrent for a few departments, most of them in the sciences.

In the end, unlike at other universities, FSU administrators balanced their budget by firing many faculty members, including many tenured professors like Froelich. That decimated the geological sciences, oceanography, and anthropology departments. “The layoffs at FSU have truly devastated faculty morale across the campus,” says anthropologist Cheryl Ward of Coastal Carolina University in Conway, South Carolina, who left FSU before the cuts. They “caused lasting harm to science programs.”

But to the FSU administration, slashing small departments that were far from supporting themselves was the only way to avoid permanently undermining education across the university. The deep, targeted cuts were unfortunately the best option, says FSU Provost Lawrence Abele. “I don't believe in cutting across the board; that weakens everything,” he says.

There's no disagreement on the 39,000-student FSU campus that the budget situation has gone from serious to dire. The big, tax-generating housing bubble burst early in Florida, which has no state income tax to cushion the loss. The Florida legislature cut the state university system's annual \$380 million budget by \$82 million over

2007 to 2010—\$55 million of it in this academic year. That budget, plus tuition, pays all salaries in the state system. And at slightly over \$3100 last year (up 15% this year), tuition at Florida state universities was the lowest in the nation, notes Joseph Travis, FSU's dean of the College of Arts and Sciences. “We're trying to run a Major League Baseball operation on a AAA or AA [minor league] budget,” he says.



Heavy FSU layoffs. The majority of faculty firings in the Florida State University System came at Florida State University (left), where about 80% of “tenure-line” layoffs occurred (right).

Across-the-board cuts “could cripple the institutional missions, starving everybody,” says Travis, so “you do elaborate cost-benefit analyses. Which of the programs are the weakest?” FSU President T. K. Wetherell made the criteria for judging the strength of a department or program explicit: student credit hours generated, degrees awarded, contract and grant expenditures, and tuition collected, all on a per-faculty as well as an absolute basis.

The state legislature funds FSU “based on enrollment,” Travis notes. The departments of geological sciences, meteorology, and oceanography came in at the bottom of 15 Arts and Sciences departments with about 6000 student credit hours per year each, according to Travis. Anthropology was fourth from the bottom with 11,000 hours; English, for example, had 50,000. “Sciences never pay for themselves,” says Travis. “There's always a subsidy arrangement” in which larger departments in effect help support smaller ones. But in the third straight

year of budget cutting, “the subsidy gets harder to find,” says Travis.

Targeted departments and programs within departments took heavy hits. Geological Sciences and Oceanography will be merged with Meteorology at the end of this academic year to form a new department of Earth, Ocean, and Atmospheric Sciences. Froelich thinks the merger is a reasonable idea but says “the university should have done it 2 to 5 years ago,” when more favorable economic conditions would have given any merged department better prospects. Anthropology barely survived elimination but will be diminished and restructured.

The numerous and often focused faculty firings have been much more controversial. Of the faculty laid off from 2007 to 2010 in the 10,700-faculty Florida State University System, 60% were laid off from FSU's approximately 1750 faculty alone, according to data collected by FSU faculty members and provided by Froelich. Approximately 43 tenured or tenure-track faculty were laid off across the system. But according to FSU English professor and Faculty Senate President Eric Walker, 35 of those 43 “tenure-line” faculty were lost at FSU.

Of about 21 tenured faculty let go across the system, all or all but one were at FSU. The College of Education was hit hard, but 10 of that 21 came out of the College of Arts and Sciences, all of them scientists in the relatively small departments of geological sciences, oceanography, and anthropology. Oceanography, at least, had just this spring received a “glowing” evaluation from the university that included an external reviewer, according to Froelich.

Elsewhere in the Florida state system, faculty fared better. At UF, “we did not cut a lot of existing people,” says Provost Joseph Glover. “We did cut a lot of vacant and newly vacant positions. And we've spread [the cuts] over a period of time, [so] this year we only have left a small amount to do.” The geological sciences department was on the block for a while, notes Perfit, its chair. The problem, as he sees it, was that “we were just small. Some of the best [geoscience] schools in the nation are being cut just because

SOURCE: P. FROELICH/FSU; E. WALKER/FSU

they're small" and would therefore seem to create only small losses to the university. His department survived, though for now the university is using stimulus money to pay everyone in the department.

FSU was the only university in the system to lean so heavily on faculty layoffs, but FSU's Travis still sees no way around that.

As to Oceanography in particular, "the discussion was never about ... value of research," he writes in an e-mail. "The discussion was always about whether we could continue to afford to subsidize" a department generating so few undergraduate credit hours and relatively few graduate degrees. And in 40 years the legislature has never

come back and restored funds it cut from the budget, he says. Other Florida universities like UF may be using stimulus money to avoid extensive faculty firings until budget cuts are restored or tuition increases accumulate, he says, but "we at FSU chose not to take this kind of chance."

—RICHARD A. KERR

IRELAND

Embryo Ruling Keeps Stem Cell Research Legal

A ruling from the Irish Supreme Court has reignited that country's debate over the legal status of human embryos, confirming the legality of research with human embryonic stem cells (hESCs) but leaving such work in a regulatory limbo that may not be resolved soon. On 15 December, the court ruled that human embryos outside the womb are not "unborn" and therefore are not protected under the country's constitution. The case before the court, in which a woman wanted to implant frozen embryos against the wishes of her estranged husband, does not directly involve stem cell research, but an opposite ruling could have made such work unconstitutional. "It's not a green light" for hESC research, says Siobhán O'Sullivan, director of the Irish Council for Bioethics. However, the ruling means "that certainly hES cell research is not banned in Ireland."

Ireland, a largely Catholic country that has experienced a growth in biomedical research over the past decade, has no laws governing human embryos outside the womb. Abortion is illegal, but assisted reproduction and research with hESCs, which are derived from lab-grown embryos, are both unregulated. Scientists have been uncertain, however, whether the Irish Constitution, which "acknowledges the right to life of the unborn," prohibits derivation of hESCs or even work with ones derived elsewhere. Public funding agencies have been similarly perplexed about whether they could fund hESC research. No such work has so far been funded.

O'Sullivan says that because of the legal vacuum, it is difficult to say if any hESC research is going on in Ireland. "If you were using them, you wouldn't want to publicize the fact," she says. But several scientists in Ireland have said they would like to work with the cells, and Science Foundation Ireland acknowledges that a few, whose names have not been revealed, have applied for public funding to do so. Frank Barry, head of the Regenerative Medicine Institute at the

National University of Ireland, Galway, says he has not submitted a grant involving hESCs, but he would welcome the chance to work with the cells. The ruling "is a major milestone for Ireland in terms of research with human ES cells," he says. "However, I think it also puts Ireland in a place where it does not want to be, where hESC research is both legal and unregulated."

In 2008, University College Cork (UCC) became the first major university in Ireland to explicitly allow work with hESCs. The univer-



Controversial move. University College Cork (above) issued regulations on research with hESCs, prompting a billboard campaign by opponents of such work.

sity's governors voted 16 to 15 to require scientists who want to work with hESCs to get approval from the university's ethics board. UCC also established a subcommittee to examine the proposed source of the cells, the goals of the intended research, and the applicant's expertise in the relevant fields. The move sparked widespread debate in Ireland, and no UCC researcher has publicly acknowledged seeking approval for hESC work.

So far, other universities have not followed UCC's lead. The Irish legislature, the Oireachtas, has also avoided dealing with the controversial issue, although ethical and law experts as well as scientists have been urging lawmakers to take action for years. A government Commission on Assisted Human Reproduction in 2005 recommended legislation to regulate fertility treatments. And in 2008, O'Sullivan's Council for Bioethics said in a paper that the government's failure to act "undermines the moral value of the embryo," and it recommended that a national regulatory body oversee embryo research.

That could be the council's final word on the subject. On 16 December, the government announced that the body would no longer be funded—a victim of severe budget cuts in Ireland. "There is now a vacuum in terms of ethical oversight," Barry says. But O'Sullivan says that the expertise that went into the council's report will still be available to politicians as they wrestle with the issue.

In the Supreme Court ruling, the judges urged the country's lawmakers to address the legal status of in vitro human embryos, calling Ireland's lack of regulation of fertility treatments "disturbing" and "undesirable." One judge cited hESC research as an example of how science has gotten ahead of the law. Health Minister Mary Harney responded by promising that the government would propose legislation in 2010 to regulate assisted reproduction, but she failed to reference hESC research.

O'Sullivan says speedy action in any case is unlikely, given that a lengthy public consultation period will be necessary. "I'd be very surprised if we have regulation in 2010," she says. But O'Sullivan hopes the process moves forward as quickly as possible. "The fact that the government has not yet regulated this area is absolutely incredible and very unfortunate indeed," she says. "It doesn't actually matter which side of the debate you sit on; what we have right now is cowboy territory."

—GRETCHEN VOGEL



In the Afterglow Of the Big Bang

Toiling behind the Iron Curtain under a tough mentor, a Russian astrophysicist uncovered secrets of the universe that have led to discoveries 4 decades later

In 1960, at the height of the Cold War, Rashid Sunyaev left his home in Tashkent, the capital of Soviet Uzbekistan, to study physics in Moscow. He was then 17 years old, with exceptional mathematical talent—the kind of student the Soviet government would have liked to groom into a weapons scientist. With genuine apprehension, Sunyaev's grandmother asked him to make a promise: Could young Rashid stay away from work that might help in the building of missiles and bombs?

Half a century later, she would have been proud of her grandson, who now directs the Max Planck Institute for Astrophysics in Garching, Germany, and is a chief scientist at the Space Research Institute in Moscow. Not only did Sunyaev manage to keep his word about avoiding secret military programs, but he also helped unlock secrets of the universe that are now pillars of modern cosmology.

Sunyaev's best-known work dates back to the late 1960s, when he and his legendary mentor—Yakov Zel'dovich, one of the fathers of the Soviet hydrogen bomb—predicted a phenomenon that causes massive clusters of galaxies to make an imprint upon the cosmic microwave background (CMB). But the impact of the so-called Sunyaev-Zel'dovich (SZ) effect is only now being fully realized. In the past decade, the effect has enabled cosmologists to measure astronomical distances precisely and determine the expansion rate of the universe, independent of other techniques such as the observation of Type 1A supernovae. And in the past 2 years, thanks to advances in detection technology, astronomers have begun using the phenomenon to discover distant clusters of galaxies. “The SZ effect has gone from being ‘Gee, good for you, Sunyaev’ to a powerful tool for probing the universe,” says

Cosmic quest. Sunyaev's contributions have revolutionized cosmology.

Robert Kirshner, an astronomer at Harvard University.

Sunyaev's scientific journey from Tashkent to the astrophysical hall of fame is one that most researchers in the West would find difficult to imagine. Although he wasn't involved in military projects, Sunyaev's early career as a researcher was constrained by the culture of secrecy and paranoia that prevailed in the Soviet Union during the Cold War. Like most of his contemporaries, he was practically forbidden from traveling outside the U.S.S.R. and didn't have access to English-language journals until a year after they were published.

In compensation, he had the mentorship of Zel'dovich, regarded as one of the most brilliant physicists of the 20th century. A father figure to his juniors, Zel'dovich inspired Sunyaev with his extraordinary analytical abilities and a dose of tough love, at times refusing to acknowledge him in the hallways if his protégé had no new results to report. “I was so lucky to meet him in my life,” Sunyaev says of the legend, who died in 1987.

The other good fortune for Sunyaev—and for astrophysics—was that the Iron Curtain did not block scientific exchanges entirely. Sunyaev's contact with a visiting scholar from the University of Cambridge helped spark the West's interest in the SZ effect, initiating a decades-long effort

by experimental physicists in the United Kingdom and the United States to validate and then exploit the phenomenon. In 2008, when a team of U.S. astrophysicists reported the first galaxy clusters discovered using the SZ effect, the result represented the bridging of two divides: between theory and application, and between two scientific cultures that until 2 decades ago had been isolated from each other by secrecy and mutual distrust.

“Useless science”

Once restricted from making trips overseas, Sunyaev is now a globetrotter, flying frequently between Garching and Moscow and speaking at conferences around the world. Last October, he flew to Austin for an invited talk at the University of Texas (UT). He got there 2 days later than planned because of a last-minute snafu: He kept the passport bearing his multiple-entry U.S. visa in a bank

locker and forgot to collect it before the bank closed for the weekend.

The incident seems consistent with Sunyaev's image as a man totally absorbed in his work, immersed in science 24/7 except when he's tending to his garden and unable to pull himself away from the computer even at mealtimes without some prodding from his wife. Sunyaev is stocky, with a square face and silver hair, and a shy manner that manifests an affable, teddy-bearish personality. One of his default expressions is of childlike wonderment, which he sported at the Texas conference while picking out greens from the salad bar and later as he listened to a talk in the auditorium, chewing the end of a pen.

Sunyaev might never have ended up as an astrophysicist if it hadn't been for a meeting with Zel'dovich in March 1965, when Sunyaev was a graduate student at the Moscow Institute of Physics and Technology planning a Ph.D. in elementary particle theory. Snowed under with coursework and itching to do real research, Sunyaev went to see Zel'dovich, who was looking for students to join his research group at the Institute of Applied Mathematics. He told Sunyaev that he would have to work on astronomy. Sunyaev, who had heard his department chair say that astronomy was "an absolutely useless science," told Zel'dovich that he'd prefer to study elementary particles. "Zel'dovich was a little amused," Sunyaev says. "He said, 'Please help me solve one or two problems in astrophysics, and after that we will work on what you want.'"

Zel'dovich's "after that" really meant "never," but it didn't matter. Sunyaev was immediately taken with astrophysics, in no small part because of Zel'dovich's brilliance and charm. "He was like mercury, very fast," Sunyaev says. "I would spend 5 days solving an equation; he would take 10 minutes to understand it."

It was a grueling apprenticeship. One in 10 students survived. "There were 4-5 of us living in a small room in the dormitory. We came only to sleep for a few hours," Sunyaev says. "You had to work in the night because the next day you had to show that you were solving problems."

In late 1965, the group received word of a discovery that would revolutionize cosmology. Researchers at Bell Labs had detected the CMB, the leftover radiation from the big bang that confirmed that the cosmos had originated as a fireball. Zel'dovich, a proponent of an alternative cold model—in which the starting point was a very low temperature—accepted right away that he had been wrong and began



Cold skies. Using the South Pole Telescope (above), John Carlstrom (right) and his colleagues have discovered new galaxy clusters revealed through the SZ effect.

suggesting ways in which this fossil radiation could be used as a marker and a backdrop for studying the evolution of the universe. "The consequences of the CMB were immediately obvious to him," says Sunyaev.

As his first assignment, Sunyaev began to work out the physics of the first few seconds after the big bang. If the universe began as a fireball, as the CMB suggested, it was a soup of photons, protons, and electrons sharing the same temperature at the moment of birth. As the universe cooled down, some of the protons and electrons came together to form hydrogen.

Working with Zel'dovich and Dima Kurt, Sunyaev calculated the rate at which this process had occurred. "It was groundbreaking

work," says Eiichiro Komatsu, a researcher at UT Austin. But the best was yet to come.

Heavy artillery

After Sunyaev completed his Ph.D. in 1966, his future was uncertain. Under government rules, he needed a special residence permit and a place to live in order to work in Moscow. "Moscow was considered paradise, so you could get permission to live there only if you agreed to do work that nobody else agreed to," he says. "Or you had to be very accomplished, like being the best ballet dancer or something."

Zel'dovich, who had been decorated with three stars from the government for his contributions to bombmaking projects, was able



to get Sunyaev a small room in an apartment shared with another family. "It was tiny compared to our house in Tashkent, but I called my mother to tell her; I was so proud," Sunyaev says.

But there was another obstacle. The chief of the KGB at the institute called Sunyaev to his office and told him to look for work elsewhere. "He said, 'Don't tell Zel'dovich why you are leaving. Just tell him you don't want to work with him,'" Sunyaev says. The reason was that his family, which had been prominent under the Tsars, was viewed as a political threat, and intelligence officials were uneasy about letting Sunyaev work at an institution where many people were working on military programs. "I said I cannot lie to Zel'dovich," he says. The KGB finally left him alone.

Between 1966 and 1970, Sunyaev worked on two fundamental ideas related to

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the CMB. The first was an analytical determination of how sound waves rippled through the fireball of radiation and matter in the moments following the big bang, shaping cosmic structure on large scales. These so-called baryonic acoustic oscillations, Sunyaev predicted, must have left a signature in the CMB. (James Peebles, an astronomer at Princeton University, reached the same conclusion independently around the same time.) The balloon-borne BOOMERANG and MAXIMA experiments finally measured the oscillations some 35 years later (*Science*, 28 April 2000, p. 595; 4 May 2001, p. 823).

The second idea is what led to the discovery of the SZ effect. In 1967 or 1968, Sunyaev heard about an astronomical mystery that had puzzled physicists since the 1930s: why the mass of galaxy clusters, as estimated from their light, seemed lower than the estimate from a measurement of the motion of galaxies within them. Although this “missing matter” is now believed to be dark matter, which puts out no light but exerts a gravitational effect on stellar motion, in the ‘60s, researchers wondered whether the missing matter could in fact be intracluster gas.

The question led astrophysicists to focus attention on the physics of this gas, and Sunyaev decided to analyze how it might interact with the CMB radiation. Every so often, he found, an energetic electron bouncing around inside the hot gas would slam into

it were a shadow of the galaxy cluster, and would be brighter at submillimeter wavelengths. Only objects as massive as clusters of galaxies could cause such a distortion in the CMB. The most exciting thing about the effect was that it was independent of redshift: The distortion in the CMB would be just as great whether a galaxy were close or far away.

Most Soviet astrophysicists were not convinced, however. At one talk Sunyaev gave in Moscow, he recalls, a white-haired professor told him the theory was nonsensical. “He said, ‘I have been teaching thermodynamics for 35 years, and I’ve never heard of radiation decreasing in brightness that way.’ I became very upset, and I was trying to explain that I have three techniques to prove this and so on, but nobody was listening to me.”

The reaction changed when Zel’dovich, late for the talk, walked in. “He said, ‘Rashid’s right and everything is correct,’” Sunyaev says with a chuckle. “And everybody began to say ‘Yes, yes, yes ... everything is correct.’ I realized what it meant to have such heavy artillery behind me.”

Worlds apart

In 1968, a 27-year-old Scottish astronomer named Malcolm Longair arrived in Moscow to spend a year with Soviet scientists as part of an exchange program between the Royal Society and the U.S.S.R. Academy of Sciences. The Royal Society

had entered. Western journals like *Nature* and *The Astrophysical Journal* routinely arrived more than a year late, with all advertisements clipped out by censors. Rarely, a recent issue would find its way to scientists with friends in the West.

But the quality of the research blew Longair’s mind. “Rashid and the others were fantastically well trained, and they were under tremendous pressure to solve problems very fast,” he says.

Another Western visitor, cosmologist R. Bruce Partridge—then at Princeton—witnessed the intensity on a visit to Zel’dovich’s house in August 1968. “There was a large room with a table in the middle and blackboards at either end,” he says. Zel’dovich himself, Partridge says, was “a Santa Claus without the beard; almost entirely bald, utterly vibrant, with shining eyes.” After a midmorning snack, the researchers grilled Partridge for 2 hours about what experimental and theoretical physicists in the United States were up to. “It was the single most draining and exhilarating experience I have ever had,” Partridge says. “It made my Ph.D. look like a walk in the park.”

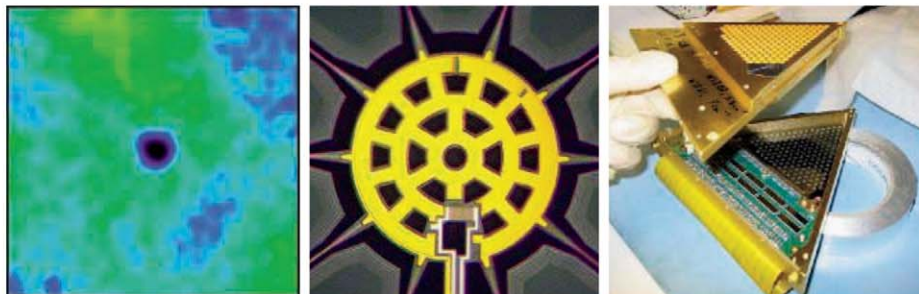
For Sunyaev, Longair was “an ambassador from another world” with a style of discussing science and writing papers that was much freer than anything he had seen. Sunyaev’s first awareness of the world of science outside the Soviet Union had come a year before, when Zel’dovich took him and some of his colleagues to an International Astronomical Union meeting in Prague. “The impact on me was unbelievable; ... it was the same as when a child goes to kindergarten for the first time and sees other children who have their own fathers and mothers,” he says. “I realized how much we were losing by being cut off.”

The isolation didn’t just prevent Sunyaev and his colleagues from learning about developments overseas; it also created hurdles in publishing both in Soviet and Western journals. All research papers authored were reviewed by special committees that would take months to approve them and would edit out words such as “nuclear.” Partridge recalls that one preprint from Zel’dovich arrived in the mail long after it had been written, with a coffee stain and crumbs on it: “Somebody had literally eaten their lunch on the paper.”

Ironically, it was the challenge of getting published in a timely way that in part prompted Zel’dovich to drive Sunyaev and the others so hard. “He told me several times that I had to identify a problem and solve it at least a year prior to my Western colleagues,” Sunyaev says.

“Everybody began to say, ‘Yes, yes, yes ... everything is correct.’ I realized what it meant to have such heavy artillery behind me.”

—RASHID SUNYAEV



Shadow in the CMB. A new galaxy cluster imaged by the South Pole Telescope as a distortion in the cosmic microwave background (left). The telescope has 966 energy detectors (center) arrayed on six wedges (right).

a CMB photon, boosting its energy and raising its frequency.

As a result, Sunyaev figured, astronomers observing a cluster would see a greater number of CMB photons above a certain frequency—around 218 GHz—and a smaller number below it. In other words, the CMB would appear fainter than normal at centimeter and millimeter wavelengths, as if

had warned him to mind his words and actions during his visit because the KGB would be watching him at every step.

A lover of Russian music and theater, Longair hit it off with Sunyaev. He met with Zel’dovich’s group often, but never at the Institute of Applied Mathematics, which was closed to outsiders. He quickly discovered what a different scientific environment he

Sunyaev was keen to have radio astronomers in Europe and the United States begin efforts to detect the SZ effect, which he knew would be a technological challenge unlikely to be met in the Soviet Union. After returning to the United Kingdom, Longair gave talks about the phenomenon at different universities and urged colleagues to try to detect it. Meanwhile, Zel'dovich heard a rumor that a Russian astronomer named Yuri Pariskii had detected the effect. Sunyaev says Zel'dovich quickly scolded him for not having written up a separate paper about the topic. They co-authored the first one in 1972.

The hunt is on

In 1976, a graduate student at Cambridge named Mark Birkinshaw decided to make the detection of the SZ effect his Ph.D. project. It was a risky topic for a dissertation; everybody knew that the effect would be difficult to detect with instruments available at the time. "I took it up because it was a brand-new thing," says Birkinshaw, now an astronomer at the University of Bristol in the United Kingdom.

Building on efforts by his thesis adviser, Birkinshaw made an attempt using the 25-meter radio telescope at Chilbolton Observatory in Hampshire. After 2 years of work on minimizing statistical noise from clouds, "I was able to detect the effect in two or three clusters, but it needed further confirmation," he says. Birkinshaw and his colleagues reported a "cast-iron" detection in 1984 with the help of the 40-meter dish at the Owens Valley Radio Observatory (OVRO) near Bishop, California.

Over the next decade, researchers got better SZ images of clusters by combining signals from multiple radio telescopes. In 1995, John Carlstrom and Marshall Joy at the California Institute of Technology made another significant improvement by mounting centimeter-wavelength receivers on the OVRO millimeter array.

Soon, Carlstrom's group and a group led by Lyman Page at Princeton were hatching plans to survey the entire sky for the SZ effect. "We decided we wanted to do SZ science big-time," says Carlstrom, who is now at the University of Chicago in Illinois.



A rare mingling. Sunyaev and Zel'dovich (right) had little contact with Western scientists. One exception was a 1968 meeting in Tbilisi, where R. Bruce Partridge, Sunyaev, Vladimir Dashevsky, and Malcolm Longair (left to right) posed for a photo.

Because millimeter- and centimeter-wavelength radiation can be absorbed by water vapor, both teams sought to find a cold, dry place with calm, clear skies. Carlstrom chose Antarctica, where his group built the South Pole Telescope (SPT)—a 10-meter instrument with an array of 966 energy detectors designed at the University of California, Berkeley, that make it many times more sensitive to the SZ effect. It saw first light in February 2007. In its first year of operation, the SPT helped spot four galaxy clusters, three of them new discoveries. Since the October 2008 results, the survey "has found many more clusters," says Carlstrom. Still more are expected from Page's group, working at the 6-meter Atacama Cosmology Telescope on Cerro Toco in northern Chile. How these clusters are distributed will help cosmologists get a better picture of large-scale cosmic evolution and answer questions about dark energy and the expansion of the universe.

After the fall

In the years following the Soviet Union's collapse in 1991, Sunyaev received job offers from institutes around the world. It



would have made perfect sense to quit, as many Russian scientists were doing, but he was reluctant to leave the Space Research Institute. "Many of my friends asked me why I would not move to the West," he says. "But I was very afraid to leave because I knew if I left, my research group in Moscow would die."

Sunyaev's acquaintances say he went to great lengths to keep his group together. In 1992, while having lunch with Partridge at a food court in Washington, D.C., Sunyaev reflected guiltily on how much the meal had cost. "He said, 'What I've just eaten here would pay a Russian scientist for a month,'" recalls Partridge, now a professor at Haverford College in Pennsylvania. On more than one occasion, he says, Sunyaev used money from his prizes to pay salaries for some of his colleagues.

Since taking his appointment at Max Planck, Sunyaev has promoted collaborations between his Moscow lab and his new institute, as well as the rest

of the world. "He is still just enormously productive and motivational," says Carlstrom. One product of those collaborations is eROSITA—an x-ray telescope being built in Germany, which will be dispatched into space on board a Russian spacecraft named Spectrum-X. The telescope's primary goal is to investigate dark energy by surveying up to 100,000 galaxy clusters.

For Sunyaev, eROSITA and other international scientific projects represent a quest for knowledge that binds humanity together. "I think astronomy is very useful," he says, contradicting his department chair's comment from 47 years ago. "What we are studying has great importance for understanding our place in the universe"—regardless of what one's place may be on the geopolitical map.

—YUDHIJIT BHATTACHARJEE



LETTERS

edited by Jennifer Sills

Bushmeat Hunting and Climate: An Indirect Link

J. F. BRODIE AND H. K. GIBBS ("BUSHMEAT HUNTING AS CLIMATE THREAT," Letters, 16 October 2009, p. 364) argue that bushmeat extraction threatens the carbon stocks of tropical forests because (i) bushmeat hunting reduces abundances of large-bodied vertebrates; (ii) tree species with large seeds reproduce poorly without large-bodied vertebrates on which they depend for seed dispersal; (iii) large seed size is correlated with high wood density in tropical trees; and (iv) trees with high wood density contribute disproportionately to the carbon stock.

Their first point is well-established, but evidence regarding the others is mixed. Killing animals reduces seed dispersal of vertebrate-dispersed trees (1–4) but does not necessarily reduce the reproduction of large-seeded trees (5), perhaps because large-bodied animals also function as seed predators and herbivores (2, 6). Likewise, the correlation between seed size and wood density in tropical trees is at best weak (7). Finally, plots with trees of higher wood density do not necessarily have higher total tree carbon stocks; depending on the site, carbon stocks may be positively related, negatively related, or unrelated to mean wood density, because of the usually countervailing effects of tree volume (8).

Lianas climbing a tropical canopy tree.



Lianas (woody vines that climb into the tree canopy) provide an alternative possible link between bushmeat hunting and carbon storage. Hunting is a disadvantage for species with seeds dispersed by animals, and therefore gives a comparative advantage to species with seeds dispersed by wind (5, 9). This strategy is much more common among liana species than trees (60 versus 20%). Liana leaves displace an equal mass of tree leaves (10), and lianas store much less carbon per leaf area than trees (11). Thus, hunting may favor lianas, and an increase in lianas is likely to reduce carbon storage.

Whatever its effect on forest carbon stores, the bushmeat crisis is unarguably a major threat to tropical biodiversity (2, 12, 13). This by itself is reason to fight it.

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Gray Wolves Not Out of the Woods Yet

IN APRIL 2009, THE U.S. FISH AND WILDLIFE Service (FWS) removed the northern Rocky Mountain population of gray wolves (*Canis lupus*) from all protections under the Endangered Species Act (ESA). Following the ESA's mandate to base listing determinations "solely on the...best scientific and commercial data available," FWS conducted an extensive analysis of regional threats to

wolves. They concluded that while "[p]ublic hostility toward wolves led to excessive human-caused mortality that extirpated the species," subsequent improvement in attitudes toward wolves ensured the long-term viability of the species.

We agree that human behaviors (and the attitudes and values underlying them) ultimately caused the extirpation of wolves in the northern Rockies, but we find little support for FWS's conclusion that attitudes toward wolves have improved, or are improving. Indeed, the

larger body of research points to the opposite conclusion (1–5). Although FWS provided more than 200 citations in their analysis, they cited just one empirical study that examined attitudes toward wolves (4). [This cannot be explained by a lack of published literature; a recent review identified 50 publications that specifically addressed the topic (6).] Thus, it appears FWS was either unaware of the extensive body of research on attitudes toward wolves, or chose to ignore this research. In fact, the only empirical article cited by FWS—

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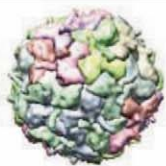
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a meta-analysis—comes to a very different conclusion: “Across the 37 attitude surveys we studied, the reported statistics were stable over the last 30 years...[t]his contradicts a recent perception among some ecologists that wolf support has recently grown” (4).

The FWS’s analysis of the threat posed by negative attitudes toward wolves is wholly inadequate. When threats to a species’ continued survival are primarily social in nature, FWS must use the same standard that goes into analyzing biological and ecological threats. It is time for FWS to expand its view of what constitutes “science” and fully incorporate the social sciences into listing determinations.

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Patents: A Threat to Innovation?

IN THE POLICY FORUM “BALANCING INNOVATION and access: Patent challenges tip the scales” (16 October 2009, p. 370), M. J. Higgins and S. J. H. Graham’s claim that Paragraph IV patent challenges are “increasingly stifling new drug innovation” is misleading.

Economists have repeatedly cautioned that correlation is not causation. The increasing number of Paragraph IV challenges, coupled with the decreasing number of FDA-approved new compounds is an interesting, but not causal, relationship. Declines in approvals could be due to a range of factors, including decreasing research productivity. Reasons for the decline in productivity include the increasing difficulty of understanding the science of more complex diseases and the focus of pharmaceutical companies on low-risk “me too” drug development (1).

Not all Paragraph IV challenges lead to early generic entry. In research documenting Paragraph IV challenges between 2004 and 2006, I found that only 13 (11%) of the 115 lawsuits resulted in a generic win (2). When

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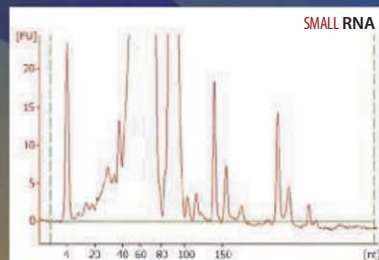
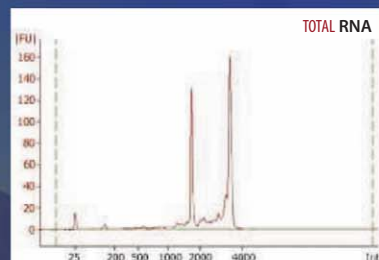
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the branded company prevails, there is neither early generic entry nor revenue loss. Perhaps more important, 73% ended in settlements, of which a subset—about 60% (3)—did not appear to result in early generic entry (2). Hence, a count of patent challenges is an unreliable indicator of losses for branded companies. The fact that so many of the lawsuits end in settlements precludes information on the patent strength and boundaries that is revealed through judicial determination.

In addition to these factors, branded pharmaceuticals are increasingly retaliating by producing or licensing authorized generics that dampen their revenue loss and the incentives for future patent challenges. For instance, in 2003, Apotex was granted the 180-day exclusivity period for its generic version of Paxil, an anti-depressant marketed by GlaxoSmithKline (GSK). Apotex had expected to generate sales of approximately \$575 million during its 6 month exclusivity, but the introduction of GSK's authorized generic lowered the actual sales to around \$200 million (4).

Paragraph IV challenges are an important mechanism for identifying patents that should not have been granted in the first place. They should be allowed to continue unless there is much more compelling evidence that they are in some way slowing innovation.

MERLINA MANOCARAN

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Response

WE AGREE WITH MANOCARAN THAT "CORRELATION IS NOT CAUSATION" and say as much in our Policy Forum's first paragraph. But Manocaran's suggestion that the pharmaceutical industry may not be facing an innovation crisis is not supported by the weight of evidence (1–3).

Companies rely critically on patents to recoup their R&D expenditures, and patent rights are only as good as the human agents (such as judges and juries) who review them.

This fact increases uncertainty for innovators, and the U.S. federal courts' weakening of patent rights over the past several years has made eventual payoffs from innovation even less likely. Added incentives offered to generic entrants in the Hatch-Waxman Act to launch Paragraph IV patent challenges, and the strategic use of this regime by generic firms, are exacerbating this problem by further undermining the market incentives to do pharmaceutical research. We are unconvinced by Manocaran's assertion that the use of authorized generics will dampen incentives to engage in Paragraph IV challenges; preliminary evidence actually suggests otherwise (4, 5).

Regarding the outcomes of Paragraph IV patent suits, the most credible evidence to our knowledge comes from a 2002 Federal Trade Commission (FTC) report cited in our article (6). Although we cannot definitively refute Manocaran's claim that 73% of a sample of cases she studied ended in settlement, we note that another researcher, using a larger sample that includes more recent cases, finds settlement rates at trial were as low as 20% and no higher than 39% during the 2006–2009 period (7). This same research shows that generics won 25% of all cases at trial (and 41% of those that did not settle), a share substantially different than the 11% that Manocaran reports (7). Moreover, we note that the 41% figure is virtually identical to the 42% generic win rate reported by the Federal Trade Commission for earlier years (6).

These inconsistencies notwithstanding, Manocaran correctly raises the issue of collusive settlements, an issue with which the competition authorities are rightfully dealing (8). However, her general observations about settlement do not affect our main thesis: The Hatch-Waxman Act is creating incentives to challenge patents and, with shifting treatments of patent law by the courts, industry revenues are increasingly threatened. In a market system, the predictable outcome without intervention will be less innovation, to the detriment of public health in the long run.

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Let Top Students Go Forth and Prosper

THE NEWS OF THE WEEK STORY "STUDY FINDS science pipeline strong, but losing top students" (Y. Bhattacharjee, 30 October 2009, p. 654) decried the "steep drop in the percentage of the highest performing students taking science and engineering jobs." But why not let these talented, scientifically trained human catalysts shift gears and move into areas such as public policy, legislation, law, finance, economics, public relations, and yes, even entertainment—that seemingly silly place where ideas and visions are formed?

It would help to have scientifically trained policy makers and legislators who truly understand the scientific and technologic issues they are voting on, with enough clout to get others on board. It would also help to have management consultants and financial analysts who avoid entrenched mindsets and realize that some "visionary" business approaches are de facto Ponzi schemes.

A protectionist attitude that expects the best students to stay within their formative disciplines has pernicious consequences. Top students in science and engineering form a gift to society—and to the scientific enterprise—when they fly forth to pollinate areas of vital importance to the public discourse. Cross-disciplinary ambassadors should be encouraged, not discouraged, if we are to build a bright new, sustainable future.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

ECOLOGY AND EVOLUTION

Leaping Lizards

Rosemary G. Gillespie

Tropical islands, vibrancy, color, and sex make for an intoxicating combination. For a biologist, if you add in high diversity, endemism, and a strikingly charismatic group of animals, it is difficult to imagine a more captivating system. In *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*, Jonathan Losos describes such a system in ardent detail. The book represents a rich compendium of information by an extraordinarily insightful biologist with a deep and broad understanding of the diversity of *Anolis* lizards in the Caribbean. Losos (an evolutionary biologist and herpetologist at Harvard University) indicates two audiences for the book: “those deeply interested in anoles and those interested in general questions of biodiversity, evolutionary biology and ecology.” I fall in the latter category, although the book certainly enhanced my appreciation of the former. The solid foundation in natural history makes this a compelling read even for biologists with a marginal interest in lizards or evolutionary biology. As Losos comments, “only by having a rich and deep understanding of the organisms we study can we have insights into how and why they vary and how they have evolved.” It is this foundation of understanding that has made books by such natural history giants as Jane Goodall, Gerald Durrell, George Schaller, and Bernhard Grzimek so influential. Although Losos’s book is aimed at a somewhat higher level, it offers the same inspiration.

One of the most intriguing aspects of the *Anolis* system is that it offers so many directions for research: Whereas some species have bizarre adaptations for crypticity, others appear to run, using speed and alacrity to elude predators and catch prey. Likewise, although much of their behavior and morphology provides mechanisms for escaping detection, the lizards display colorful dewlaps and perform staccato head bobbing to signal to mates and competitors. Clearly, sexual selec-

Lizards in an Evolutionary Tree
Ecology and Adaptive Radiation
of Anoles

by Jonathan B. Losos

University of California Press,
Berkeley, 2009. 527 pp. \$75, £52.
ISBN 9780520255913. Organisms
and Environments.

tion can run counter to natural selection—but it doesn’t always. Indeed, anoles display key innovations in the form of toe pads that allow them to explore a new ecological arena through natural selection and dewlaps that may enhance the rate of speciation through sexual selection. Perhaps the most intriguing element in the sys-

tem is that, despite the apparent diverse selective pressures acting on these lizards, there is remarkable predictability in the repeated evolution of ecomorphs on the Greater Antilles.

Except for a brief discussion in the final chapter of parallels in other systems, Losos quite wisely makes little attempt to analyze the lizards in the context of other studies: Had the book taken this avenue, it would have grown to encyclopedic proportions [like those of (*J*)]. However, readers always draw parallels to systems with which they are most familiar. So I will mention some of these in the context of radiations in the Hawaiian Islands, because such comparisons allow us to evaluate common themes. For example, in the anoles, “body size diverged early in the radiation without much subsequent change, but ... habitat use has been diverging throughout the radiation.” In the same way, Hawaiian sap-feeding planthoppers in the genus *Nesosydne* (Delphacidae) appear to have undergone extensive ecological shifts early in their radiation and relatively minor changes subsequently (2).

In the context of community assembly, the very clear pattern of repeated evolution of ecomorphs in the Caribbean anoles suggests that evolution has allowed species to occupy the ecological space more rapidly than has colonization. In contrast, many Hawaiian plants underwent major ecological changes early in their radiation, with communities on younger

islands being filled simply by colonization of ecological equivalents from older islands. For these plants, it appears that colonization occurs more readily than evolutionary shifts within an island. Yet, in other lineages (both plants and animals), there are varying levels of independent ecological radiation into the diverse habitats on each island (3).

Repeated evolution of ecomorphs with discrete sets in any one habitat, as in the anoles, is most pronounced in spiders (genera *Tetragnatha* and *Ariamnes*), and comparisons are intriguing. First, as in anoles, some habitats may be missing a member (usually the same one) of the ecomorph set. Losos considers the most likely explanation for this to be island size. The geochronology of the Hawaiian Islands may allow the phenomenon to be scrutinized in some detail. Second, the anole radiation is characterized by several taxa with unique ecological attributes. In Hawaiian *Tetragnatha*, taxa outside the “spiny leg” clade have several unique representatives, and convergence in the more-encompassing lineage seems—if anything—to be limited to the form



Uniquely variable. *Anolis distichus*, a widespread and highly polymorphic trunk anole, exhibits substantial intra- and interpopulational variation in dewlap color (here *A. d. vinosus*, from the Tiburon Peninsula, Haiti).

of the web rather than the body phenotype. Third, anole ecomorphs have arisen almost entirely through convergent evolution, whereas in Hawaiian *Tetragnatha* spiders, communities have been filled by a combination of colonization and evolution. The domination of evolution over colonization in the anoles (perhaps also in Hawaiian *Ariamnes* spiders) indicates that movement in the anoles must be severely curtailed, an attribute that Losos also discusses in the context of population structure.

Lizards in an Evolutionary Tree offers a winning combination of enchanting animals

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and an idyllic setting that addresses some key biological questions. Losos documents an extraordinary history of research on almost every imaginable attribute of *Anolis* lizards. He frequently stops to take stock before presenting the hypotheses and asking how these could be tested, and he whets our appetites by presenting avenues for future study. What an exciting time it is for evolutionary biology, and anoles provide one of the most compelling systems to further our understanding of the field.

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10.1126/science.1182503

COMMUNICATING SCIENCE

If Our Messages Are To Be Heard

Peter Kareiva

Scientists know important things. They know about the role of greenhouse gases in global warming. They know how genes are inherited. They know how the body fights off infections. They know that the world's ecosystems are being needlessly degraded. But most scientists do not know how to talk to anyone other than scientists. As a consequence, political leaders and the public at large either ignore or, perhaps more accurately, are bored by whatever it is that scientists are trying to tell them. The general population's attitude toward climate change has become the iconic story of a public that pays no heed to the message of scientists. This inability of scientists to connect with the nonscientists has far-reaching consequences well beyond any single issue such as global warming. Randy Olson and Cornelia Dean have written two very different books with the same goal: to school scientists on how to communicate with and reach the public.

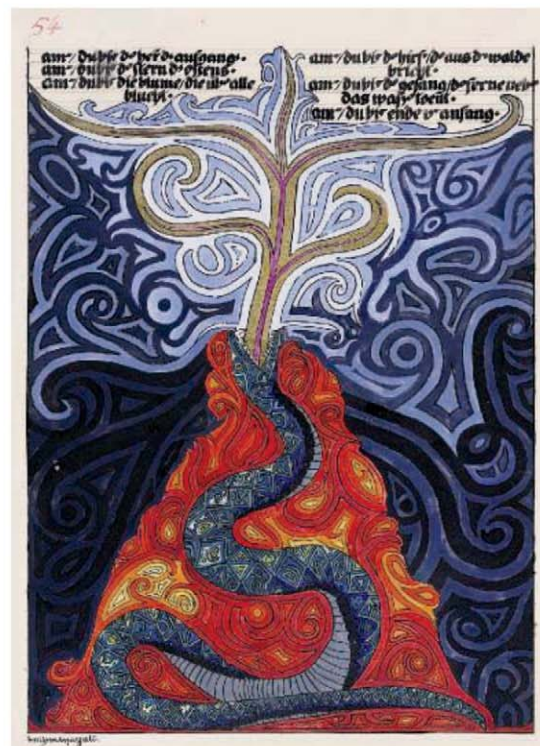
Dean, formerly a science editor for the *New York Times*, knows well how caveats kill the message. And she has seen firsthand the freezing out that instantly accompanies even

BROWSINGS

The Red Book of C. G. Jung. Rubin Museum of Art, New York, through 15 February 2010.

On the lower level of the Rubin Museum of Art in New York, there is a small exhibition, "The Red Book of C. G. Jung: Creation of a New Cosmology." Jung, who was a highly influential figure in the history of psychology and psychoanalysis, spent a period of time during World War I in self-investigation and waking visions. He created phantasmagorical, multicolored, and detailed images that illuminate his description of this exploration in the recently released *Liber Novus* (commonly known as "The Red Book"). The beautiful images, as well as a video describing him by guest curator Sonu Shamdasani, are shown in the exhibit. During the exhibit, the museum has been sponsoring a series of dialogues between notable individuals (including Twitter co-founder Jack Dorsey and philosopher Cornel West) and psychoanalysts; audio podcasts of many of these dialogues will be available at www.wnyc.org. The museum is also showing a film series based on Jungian themes.

—Barbara Jasny



a hint of patronizing utterances. As a journalist who was in at the founding of the Tuesday "Science Times," Dean saw thoughtful media coverage of science initially grow but then dwindle under the fiscal pressures of failing newspapers. *Am I Making Myself Clear?* is as much about why scientists need to talk to the public as it is about how we should talk science to the public. She argues that scientists need to develop a civic persona that finds some way to communicate science.

Dean's wisdom, especially for engaging in the political arena, is delivered with a mix of authority and charm, as is evident in her advice on how to respond to questions from a congressional committee or staffer: "Say 'I don't know' when appropriate and offer to provide the needed information later. But as the old saying goes, don't let your mouth write checks your ass can't cash. If you promise to provide additional information, memos, or the like, be prepared to produce them, and fast."

Blogs and e-mail campaigns have become hugely influential—for spreading information,

creating their own news, and building a community of like-minded activists. However, as Dean cautions, the work required for maintaining an effective blog is enormous, and the return on investment from a scientist's perspective may be too low. The solution may well be science collectives that maintain blogs and can respond instantly to the latest story about a child dying from a flu vaccine or some article that purportedly overturns 30 years of global circulation models. But before we give ourselves over to the Internet, Dean reminds us what we all know—there is too much information out there, so the key is to master the arts of standing out above the confusion and delivering a message that is heard, understood, and remembered. This is hard enough for a captive audience in a classroom and orders of magnitude harder when trying to reach a public audience that has many vibrant options for reading, viewing, and listening. Yet parents with teenagers in their household will have some idea of the power

Don't Be Such a Scientist Talking Substance in an Age of Style

by Randy Olson

Island Press, Washington, DC, 2009. 215 pp., illus. Paper, \$19.95. ISBN 9781597265638.

Am I Making Myself Clear? A Scientist's Guide to Talking to the Public

by Cornelia Dean

Harvard University Press, Cambridge, MA, 2009. 284 pp. \$19.95, £14.95, €18. ISBN 9780674036352.

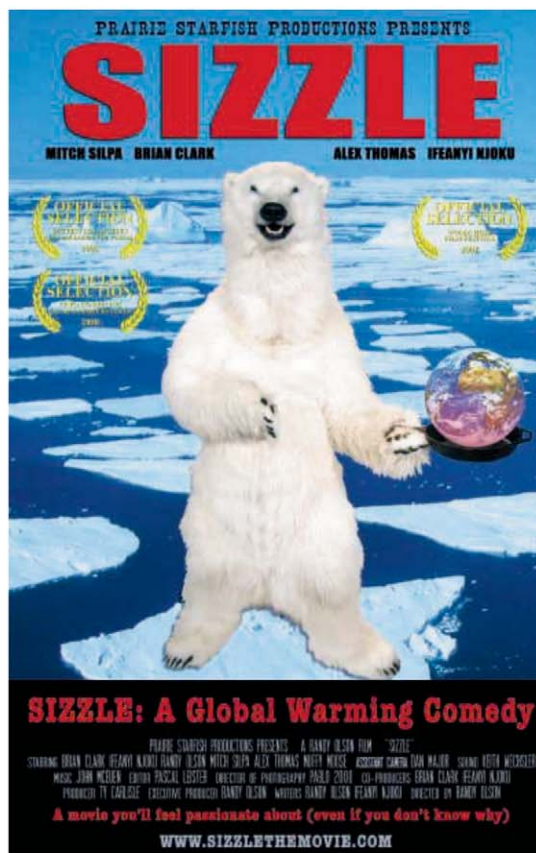
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of YouTube postings that “go viral” and suddenly become talked about in every high school in the country, having been viewed by millions. The Internet can be a powerful means for communication, and scientists need to better tap into it.

Olson’s *Don’t Be Such a Scientist* is also about reaching the public in fresh ways, in particular through movies and the entertainment industry. Although his writing style is irreverent and much more raw than Dean’s elegant prose, Olson’s insights are equally valuable. They come partly from his having lived in the academic world for much of his life. He was a marine biology professor who gave up his tenured university position to go to Hollywood and learn how to make movies. Olson’s latest film, *Sizzle: A Global Warming Comedy*, uses goofy humor to inform nontechnical audiences about global warming. Olson’s shtick is that science must join the 21st century and reach people where they live—in a world of celebrities, videos, and movies. Olson advocates using entertainment to convey scientific content, and he emphasizes the need to reach people in their hearts and guts (and maybe even their groins). Some readers will find Olson’s autobiographical treatise off-putting and a bit narcissistic. But to be turned off by Olson’s style only proves his point. Get with it. Film and visual images have enormous capacity to tell stories and change thinking.

The traditional mode of communicating science is not working; surveys that probe the public’s mastery of basic scientific issues consistently document that scientists are failing to reach the public (1). Stuffy and dry science is a losing proposition. Olson recommends that researchers experiment with new approaches, take risks, develop their own voices, and above all recognize the power of storytelling. Whereas social scientists, linguists, and political scientists might advise us how to better frame the issues, these “ists” are not where Olson turns for inspiration. His book is a plea for indulging one’s artistic nature in pursuit of more heartfelt connections to the public. That message will make many scientists squirm, especially those who take refuge in the caricature of science as objective, fact-based, and free from personal values. If scientists were seen as adventurers and explorers instead of as fact-mongers and talking encyclopedias, people might stay awake long enough to learn their science lessons.



Olson is at his best while recounting how unlikeable scientists can be with their relentless critical thinking, negativity, and smarter-than-thou condescension. A particularly telling anecdote concerns a public debate in New York City between two teams arguing whether or not global warming is a crisis. When the moderator asked the “global warming is a crisis” team why it thought the other side was misrepresenting the issues, one scientist responded, “I don’t think they [“the global warming is not a crisis” team] are completely doing this on a level playing field that the people here will understand.” With that statement, the researcher insulted and instantly alienated his highly educated Manhattan audience. Before-and-after polling revealed that, as a result of watching the debate, the audience (which, admittedly, had been stacked by the organizers) had shifted its position by 16 percentage points against the “global warming is a crisis” view.

It is not hard to figure out why Olson, Dean, and others (1) are in 2009 tackling the cultural and communication divide between science and the rest of humanity. Scientists everywhere are bemoaning popular misunderstandings regarding global warming, stem cell research, and childhood vaccination programs, to name just a few topics where science intersects public policy. Fifty years ago, C. P. Snow gave a famous warning

“an exceedingly clever vehicle for making science engaging”
—Variety

about the dangerous divide between science and the humanities, a divide that he thought put human destiny at risk (2). Today Snow’s warning is even more pertinent, and yet scientists continue to be resoundingly inept at reaching the public. Both Dean and Olson mention that Carl Sagan was spurned by the National Academy of Sciences, purportedly because he was too successful a communicator. The professional reward system in science routinely belittles the “media scientist” or the “advocate scientist.” One senses that this is beginning to change, but scientists still have a great deal to learn about effective communication.

Dean and Olson both underemphasize the single biggest reason why scientists are often such ineffective communicators. The failure of scientists as communicators is that they do not know how to listen, especially when it comes to the “uneducated public.” Brilliant scientists can be stunningly dumb when it comes to dealing with people. I recall one world-renowned ecologist who nearly caused a brawl in a Pacific Northwest tavern by preaching to the bartender about the extinction crisis and self-righteously scolding the tavern for advertising “fried spotted owl” on the bar menu. Instead of trying to understand the values and thinking behind attacks on the Endangered Species Act, global warming, or the theory of evolution, scientists too often deride what they see as an ignorant public, with potentially devastating consequences (3). The foundation of successful communication is listening to and respecting your audience. *Don’t Be Such a Scientist* and *Am I Making Myself Clear?* ought to be required reading in all science graduate programs, but they should be supplemented with the wisdom of Nelson Mandela, who knew how to reach a public that initially vilified him (4). Scientists could learn from Mandela that to win people’s minds you must first get them to listen, and people will listen only if they feel that they are respected.

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10.1126/science.1183465

Opportunities for Research and NIH

Francis S. Collins

The mission of the National Institutes of Health (NIH) is science in pursuit of fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to extend healthy life and to reduce the burdens of illness and disability. The power of the molecular approach to health and disease has steadily gained momentum over the past several decades and is now poised to catalyze a revolution in medicine. The foundation of success in biomedical research has always been, and no doubt will continue to be, the creative insights of individual investigators. But increasingly those investigators are working in teams, accelerated by interdisciplinary approaches and empowered by open access to tools, databases, and technologies, so a careful balance is needed between investigator-initiated projects and large-scale community resource programs. For both individual and large-scale efforts, it is appropriate to identify areas of particular promise. Here are five such areas that are ripe for major advances that could reap substantial downstream benefits.

High-Throughput Technologies

In the past, most biomedical basic science projects required investigators to limit their scope to a single aspect of cell biology or physiology. The revolution now sweeping the field is the ability to be comprehensive—for example, to define all of the genes of the human or a model organism, all of the human proteins and their structures, all of the common variations in the genome, all of the major pathways for signal transduction in the cell, all of the patterns of gene expression in the brain, all of the steps involved in early development, or all of the components of the immune system. Further development of technologies in areas such as DNA sequencing, imaging, nanotechnology, proteomics, metabolomics, small-molecule screening, and RNA interference are ripe for aggressive investment. Furthermore, these technologies will spur the production of massive and complex data sets and will require major investments in computational biology.

As one example, the Cancer Genome Atlas (1) is now poised to derive comprehen-



sive information about the genetic underpinnings of 20 major tumor types. This information will likely force a complete revision of diagnostic categories in cancer and will usher in an era where abnormal pathways in specific tumors will be matched with the known targets of existing therapeutics. Another example is the opportunity to understand how interactions between ourselves and the microbes that live on us and in us (the “microbiome”) can influence health and disease (2).

Translational Medicine

Critics have complained in the past that NIH is too slow to translate basic discoveries into new diagnostic and treatment advances in the clinic. Some of that criticism may have been deserved, but often the pathway from molecular insight to therapeutic benefit was just not discernible. For many disorders, that is now changing. Three major factors have contributed to this: (i) the discovery of the fundamental basis of hundreds of diseases has advanced dramatically; (ii) with support from the NIH Roadmap, academic investigators supported by NIH now have access to resources to enable them to convert fundamental observations into assays that can be used to screen hundreds of thousands of candidates for drug development; (iii) public-private partnerships are being more widely embraced in the drug-development pipeline to enable biotech and pharmaceutical companies to pick up promising compounds that have been effectively “de-risked” by academic investigators and to

The promise of fundamental advances in diagnosis, prevention, and treatment of disease has never been greater.

bring them to clinical trials and U.S. Food and Drug Administration (FDA) approval.

As one example, the NIH Therapeutics for Rare and Neglected Diseases (TRND) (3) program will allow certain promising compounds to be taken through the preclinical phase by NIH, in an open environment where the world's experts on the disease can be involved. Furthermore, as information about common diseases increases, many are being resolved into distinct molecular subsets, and so the TRND model will be even more widely applicable.

The first human protocol (for spinal cord injury) involving human embryonic stem cells (hESCs) was approved by the FDA in 2009, and the opening up of federal support for hESC research will bring many investigators into this field. The capability of transforming human skin fibroblasts and other cells into induced pluripotent stem cells (iPSCs) opens up a powerful strategy for therapeutic replacement of damaged or abnormal tissues without the risk of transplant rejection (4–6). Although much work remains to be done to investigate possible risks, the iPSC approach stands as one of the most breathtaking advances of the last several years, and every effort should be made to pursue the basic and therapeutic implications with maximum speed.

Benefiting Health Care Reform

U.S. expenditures on health care now represent 17% of our Gross Domestic Product, are continuing to grow, and are excessive as a percentage of per capita gross income com-

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pared with other developed countries. Yet few would argue that the quality of care is what it should be. Reinventing health care is thus an urgent national priority, and NIH can make substantial contributions. Among projects that must be pursued are the following.

Comparative effectiveness research. NIH has supported clinical studies for many years that evaluate outcomes of medical treatment options. For example, the Diabetes Prevention Program (7) demonstrated substantially better benefits of exercise and life-style changes over medication in preventing the onset of diabetes. The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study (8) compared older, cheaper antipsychotic drugs with newer ones, demonstrating that the older drugs worked just as well and had a better side-effect profile. With support from the American Recovery and Reinvestment Act (ARRA), NIH is investing \$400 million in such studies in fiscal year (FY) 2009–FY 2010 and expects to continue high levels of support.

Prevention and personalized medicine. Advances in pinpointing individual genetic and environmental risk factors for disease now make it possible to focus prevention strategies more effectively. For example, research to establish the utility of information about individual genetic risks associated with breast cancer, colon cancer, or prostate cancer may help inform the timing of mammography, colonoscopy, or prostate-specific antigen screening. Also, both retrospective and prospective analyses of how individual information about disease risk actually alters health behaviors and clinical outcomes will be critical.

Health disparities research. The health of racial and ethnic minorities, people living in poverty, and other disadvantaged groups in the United States is substantially worse than the health of the overall population (9). Any successful reform of the health-care system will require attention to these groups. Using new and powerful tools to disaggregate environmental and genetic contributions, NIH will seek to pinpoint causes of health disparities and to point the way toward solutions.

Pharmacogenomics. There is compelling evidence of a correlation between genotype and drug response for more than a dozen drugs (10), and that number is growing. Prospective studies will be needed for many of these applications, if FDA is to be convinced that genotyping should be required on the label and if insurance companies are to be persuaded to reimburse for the cost of genotyping.

Health research economics. Although the major justification for biomedical research will always be the relief of human suffering and the

prolongation of life, further precision is needed in assessing the economic value of research initiatives, especially those that are large and expensive. Models that attempt to capture this cost-benefit balance in Disability Adjusted Life Years (DALYs), Quality Adjusted Life Years (QALYs), Value of Investment approaches, or other metrics are only partially successful in providing the kind of information that policy-makers need. NIH plans to initiate a grants program to encourage development and application of more rigorous models.

Focusing More on Global Health

Much of recent global health research has justifiably been focused on AIDS, tuberculosis, and malaria (11). It is also critical to go beyond the focus on the “big three” diseases to neglected tropical diseases of low-income countries that contribute to staggering levels of morbidity and mortality. In collaboration with other sources of support such as the Bill and Melinda Gates Foundation, NIH can play a major role in ramping up the discovery of novel targets in both pathogen and host and work to facilitate advances in prevention, diagnostics, and therapeutics. Helping to build capacity and training opportunities in the developing world will be a critical component of such progress. Additional resources will also be needed to respond to the growing challenge of chronic noncommunicable diseases and injuries.

Reinvigorating and Empowering the Biomedical Research Community

The U.S. biomedical research community has been under stress since the flattening of the NIH budget in 2003 and may potentially face even more severe disruptions at the end of ARRA funding in FY 2011. Looking toward the future, a critical feature must be an emphasis on innovation. Although the two-level NIH peer-review process is much admired and much copied around the world, its potential tendency toward conservatism is a chronic concern and invariably worsens when funding is very tight. Recognizing these problems, NIH announced a series of concrete steps in June 2008 to enhance the peer-review process (12). Effects of these new steps will be closely monitored, and additional reforms to encourage innovation will be undertaken as needed. Meanwhile, it will be critical to resist political attacks on certain areas of sensitive research (such as drug abuse and sexually transmitted diseases); peer review should remain the appropriate standard for making funding decisions.

The success of biomedical research rests squarely on the robustness of NIH training

programs for the next generation of basic and clinical scientists. These training programs face many challenges: (i) the number of supported positions is insufficient to support all of the best applicants; (ii) stipends for graduate students have failed to keep up with inflation; (iii) the relative paucity of new faculty positions over the last few years has forced many talented scientists to remain for long periods in postdoctoral positions; (iv) the typical age at which an investigator obtains his or her first independent NIH grant support has risen to 40 or older; (v) training programs to encourage underrepresented minority participation have thus far generally failed to generate a scientific workforce that resembles the rest of the nation. Solutions in all these areas are badly needed. One initiative that could encourage earlier independence of the most talented young scientists would be a program modeled after the Whitehead Institute Fellows program, where carefully chosen scientists who have just obtained Ph.D., M.D., or M.D.-Ph.D. degrees are provided with laboratory space, technical support, financial resources, and senior mentorship, but are allowed to pursue independent projects, effectively skipping over 5 years or more of postdoctoral training.

Finally, it is time for NIH to develop better models to guide decisions about the optimum size and nature of the U.S. workforce for biomedical research. A related issue that needs attention, though it will be controversial, is whether institutional incentives in the current system that encourage faculty to obtain up to 100% of their salary from grants are the best way to encourage productivity.

Recruiting, retaining, and empowering scientists from many disciplines to work together, supported by a stable trajectory for biomedical research support, are critical to realize the unprecedented opportunities that lie in front of us. It is time to be bold.

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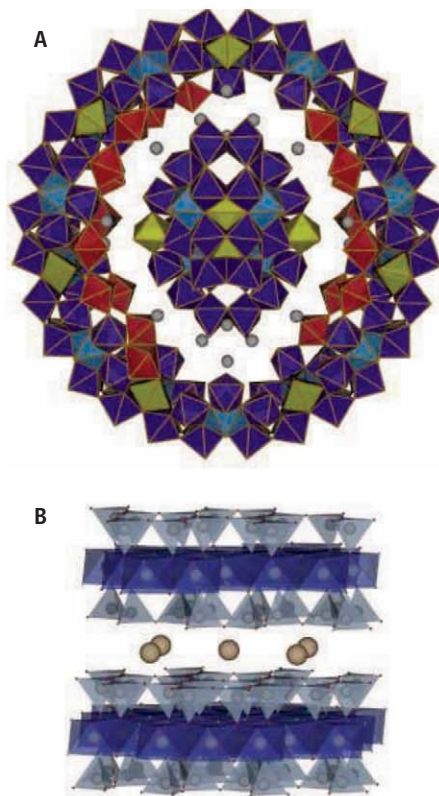
Molecular Donuts and Donut Holes

Kenton H. Whitmire

The molecular host-guest complex reported by Miras *et al.* on page 72 of this issue (1) provides insight into a remarkable system in which a template molecule forms, builds a host around itself, and then exits once its job is done. By using a flow reactor system, the authors capture an early stage in this process, where a molecular donut has its “hole” still inside (see the figure, panel A). In this complex, the two metal oxide anions are held together by a ring of Na^+ ions and hydrogen bonds. The success of this approach suggests possibilities for finding other transient host-guest complexes and designing new open framework structures.

At a donut shop, you might have the opportunity to buy not only donuts but some donut “holes” as well. So if you encounter a molecule or ion with a large void within it, you might ask yourself what happened to the “molecular donut hole” around which it formed. This is exactly the situation encountered by Miras *et al.* In a synthetic tour de force, the authors isolated an elliptical, flattened donut-shaped ion containing 150 metal atoms, with the template that led to its formation still present (see the figure, panel A). Wheel-like structures have been known for some time, but understanding how they formed has been left to speculation (2, 3).

The idea of creating molecules that selectively encapsulate other molecules or ions (4, 5) is now known as supramolecular chemistry. Widely used in chemistry, chemical engineering, biology, and materials science, it exploits secondary intermolecular interactions such as hydrogen bonding to direct molecular structure and reactivity. Not only are molecules designed to encapsulate specific molecules or ions, but molecules or ions also serve as templates for creating intricate structures around themselves. Complex molecular architectures with tailored physical and chemical properties have been synthesized in this way. For example, the size and shape of the cavities in zeolites (microporous silicon and aluminum oxide materials) can be determined by the presence of a templating molecule or ion that can then be removed, leaving the porous architecture intact (6). Similarly, tissue engineering makes use of molecular scaffolds to template the growth



Getting ready to leave. The host-guest complex reported by Miras *et al.* (1) (A) is stabilized by sodium atoms (gray) on the surface of the guest molecule. Addition of more electrons leads to elimination of the guest molecule. A similar situation is found in mica (B), a layered mineral that cleaves easily along the planes of the intercalating ions. For color coding in panel A, see (1).

of tissues and organs. Supramolecular assemblies determine the structures and functions of proteins, DNA, and cell membranes, and the principles that make them work so effectively can be employed in vitro to solve non-biological problems in materials design or catalysis. For example, a designed molecule containing four iron atoms connected by organic molecules into a tetrahedral cage can reversibly encapsulate highly reactive P_4 molecules, effectively producing a “molecular bottle” that isolates the molecules from the atmosphere, in which they would burn spontaneously (7, 8).

Miras *et al.* describe the structure of $\text{Na}_{22}[\text{Mo}_{36}^{\text{VI}}\text{O}_{112}(\text{H}_2\text{O})_{16}] \subset [\text{Mo}_{130}^{\text{VI}}\text{Mo}_{20}^{\text{V}}\text{O}_{442}(\text{OH})_{10}(\text{H}_2\text{O})_{60}] \cdot 180\text{H}_2\text{O}$, abbreviated as $\text{Na}_{22}\{\text{Mo}_{36}\} \subset \{\text{Mo}_{150}\} \cdot 180\text{H}_2\text{O}$. They obtained the molecule by carefully controlling the degree

A transient template steers the self-assembly of a “giant” metal oxide wheel.

of reduction of a sodium molybdate solution in a flow system. The $\{\text{Mo}_{36}\}$ guest ion is known to form spontaneously in acidified solutions of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. The oxidation state of Mo does not change, and consequently no reducing agent is needed to produce it. Under such conditions the $\{\text{Mo}_{150}\}$ host cannot form, because it requires the addition of 20 electrons, which are provided by introducing the reducing agent sodium dithionite.

Given that both host and guest are negatively charged ions, one might think that electrostatic repulsions would prevent the system from being stable. However, this problem is circumvented by the binding of Na^+ ions between the $\{\text{Mo}_{36}\}^{8-}$ and $\{\text{Mo}_{150}\}^{14-}$ ions. The host can thus effectively be viewed as forming around a positively charged $[\text{Na}_x\{\text{Mo}_{36}\}]^{(x-8)+}$ rather than a negatively charged ion. This is not unlike the structure of mica, where slabs of aluminum and silicon oxide are separated by layers of metal cations such as sodium, magnesium, or calcium (see the figure, panel B). Some uses of mica come from the ready cleavage of the layers along the planes of the intercalating ions. A similar situation occurs in the host-guest complex described by Miras *et al.*, where cutting the ions apart is initiated by addition of eight more electrons to $\{\text{Mo}_{150}\}$. As a result, the electrostatic interactions become overwhelming, and the templating guest is eliminated. Both the $\{\text{Mo}_{36}\}$ template/guest and the $\{\text{Mo}_{150}\}$ host can be isolated independently after the reduction process is complete.

It is not necessary to have a template involved in the formation of a hollow structure. In some cases, the bond lengths and angles for different metal ions in an oxide structure will lead to spontaneous curvature that can cause the structure to close on itself, giving a ring, sphere, or tube. Aluminosilicates accomplish this in nature by bringing together the appropriate ratio and arrangement of Si^{4+} and Al^{3+} ions; the slightly different bonding requirements of the two ions cause the array to curve spontaneously into allophane (9) or imogolite (10, 11)—spherical and tubular metal-oxide analogs to fullerenes and carbon nanotubes, respectively. In the present case, not only is the curvature of the $\{\text{Mo}_{150}\}$ guest facilitated by the presence of the template, but it is also stabilized by the molybdenum ions adopting two different oxidation states, Mo^{6+} and Mo^{5+} .

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The discovery of an ephemeral guest template that forms its host and then leaves will no doubt prompt others to look for “molecular donut holes.” The findings suggest new strategies for creating other open structures by judicious combinations of ions of different metals and/or the same metals in differing oxidation states.

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ASTRONOMY

Serendipitous Astronomy

Kenneth R. Lang

Four hundred years ago, Galileo Galilei turned his newly constructed spyglass to the skies, and thus began astronomers’ use of novel telescopes to explore a universe that is invisible to the unaided eye. The search for the unseen has resulted in many important unexpected discoveries, including Jupiter’s four large moons, the planet Uranus, the first asteroid Ceres, the large recession velocities of spiral nebulae, radio emission from the Milky Way, cosmic x-ray sources, gamma-ray bursts, radio pulsars, the binary pulsar with its signature of gravitational radiation, and the cosmic microwave background radiation. The observable universe is a modest part of a much vaster, undiscovered one that remains to be found, often in the least expected ways (1, 2).

A few months after presenting the Venetian Doge a spyglass, or telescope, as a valuable tool of war, Galileo turned a telescope of his own making to the nearly full Moon, and on 7 January 1610, Jupiter “appeared” to him (3, 4). As luck would have it, the planet was then just above the Moon and the second brightest object in the sky (see the figure). Three, then four, “Medicean stars” were found accompanying Jupiter, orbiting the planet at speeds that decreased with distance from it. No one had predicted the possible existence of moons orbiting Jupiter.

In the next century, two other unanticipated discoveries occurred when astronomers were observing stars with telescopes of then-unsurpassed quality. William Herschel was using his reflecting telescope to survey bright stars, locating nearby faint stars that might help determine the distances of the bright ones. Giuseppe Piazzi was using a finely calibrated instrument to compile a catalog of accurate star positions. Herschel discovered

Uranus on 13 March 1781 (5), and Piazzi found the first asteroid, Ceres, on 1 January 1801 (6); both objects moved against the background stars and were initially thought to be comets.

In the early 20th century, Vesto Slipher unexpectedly helped us move beyond the stars into the expanding universe. Working at the Lowell Observatory in Flagstaff, Arizona, he was measuring the rotations of spiral nebulae, whose bright centers were then thought to be newborn stars—the surrounding spiral arms had been interpreted as nascent planetary systems. Using a spectrographic camera with a modest 24-inch (0.61 m) refractor telescope, he found, in 1917,

Many of the seminal discoveries in astronomy have been unanticipated.

that the outward velocities of 25 spiral nebulae were well in excess of the velocity of any known cosmic object. Almost all of the spiral nebulae were moving away from the Earth, at astonishingly high velocities, up to 1100 km s⁻¹. This suggested to Slipher that the spiral nebulae were stellar systems, or “island universes.” By 1929, Edwin Hubble showed that the measured distances, established by him using the superb light-gathering power of the 100-inch (2.5 m) Hooker telescope on Mount Wilson, were roughly correlated with Slipher’s velocities. This relationship is now attributed to the expanding universe, which no one had anticipated at the time Slipher made his measurements.



Looking up. A rendering of how the night sky of Padova, Italy, would have looked on 7 January 1610, 6:30 p.m., looking east.

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Other serendipitous discoveries revealed an entirely different, explosive universe, which was unveiled when new technologies let us capture invisible forms of radiation, first as radio waves and then as x-rays and gamma rays. Radio noise of cosmic origin was, for example, inadvertently found in 1933 by Karl Jansky, working for the Bell Telephone Laboratory to determine the sources of interference with terrestrial radio communications. He built a rotating radio antenna that pointed sideways at the horizon instead of up into the sky. Fortunately, the broad field of view, of 25°, occasionally included the Milky Way, which produced an “interstellar interference” comparable in intensity to lightning discharges in distant thunderstorms.

The new field of radio astronomy nevertheless had to await the development of radio and radar technology during World War II and the subsequent construction of radio interferometers to provide angular resolving power. The radio galaxies that were then discovered emit unanticipated radio power, equivalent to the visible light of a million million (10^{12}) stars which is attributed to electrons moving at nearly the speed of light.

Enormous amounts of energy are also released from cosmic x-ray sources, which were discovered during a 5-min rocket flight primarily designed to detect x-rays from the Moon. As x-rays are absorbed in our atmosphere, cosmic x-ray sources must be observed with instruments launched above the obscuring air, in rockets or satellites. By the mid-20th century, such brief rocket flights had shown that the Sun radiates detectable x-rays, and it was thought that lunar material might also emit them when illuminated by solar x-rays. Riccardo Giacconi's group at the American Science and Engineering Company (AS&E) tried to detect the Moon's x-rays in 1962. Instead, they found evidence for the first known, discrete x-ray source outside the solar system.

The x-ray emission of any Sun-like star would be far too faint to be detected with existing equipment, due to the stars' greater distances. Nevertheless, the AS&E group intended to search for other unknown cosmic x-ray sources. The consequences of their resulting discovery were enormous, leading to the development of focusing x-ray telescopes placed aboard a host of orbiting x-ray observatories and to the discovery of new cosmic objects, such as million-degree gas spiraling into stellar black holes.

The most energetic sources found in the universe, at least so far, are the gamma-ray bursts whose duration is measured in seconds and which never reappear in exactly the same part of the sky. Their discovery was the unex-

pected result of defense satellite observations designed to detect clandestine nuclear bomb explosions in Earth's atmosphere, on the Moon, or in outer space. As reported by R. Klebesadel, I. Strong, and R. Olson in 1973 (7), brief, intense gamma-ray bursts were instead found to be coming from the distant cosmos. The long-lasting afterglow of the cosmic gamma-ray bursts was eventually detected at visible wavelengths, enabling the distances to be determined by spectroscopy. We now know that some of these bursts radiate, for a few seconds, gamma-ray energy equivalent to the visible-light energy emitted by all the galaxies in the observable universe.

Pulsars were discovered during a survey of the scintillations, or “twinkling,” caused when radiation from cosmic radio sources passes through the Sun's winds. To study this effect, Antony Hewish and his colleagues at Cambridge University built a large array of 2048 dipole antennas connected to a radio receiver and chart recorder with a time constant of 0.1 s, the time scale of the scintillations. Graduate student Jocelyn Bell found a source of strong radio scintillation fluctuating in the middle of the night, when the array was pointed away from the Sun and the effects of the solar wind should have been small. Further investigations led to the detection of periodic radio pulses, repeating with an exceedingly precise repetition period of 1.3372795 s. Within months, other large radio telescopes were used with rapid time sampling, rather than the long integration times formerly used to detect faint cosmic radio signals, resulting in the discovery of many more pulsars. No one had foreseen either the existence of pulsars or their subsequent interpretation as rotating neutron stars with intense, beaming magnetic fields.

The first binary radio pulsar, designated PSR 1913+16, was found in 1975 by Russell Hulse and Joseph Taylor as the result of a high-sensitivity, computerized search for new radio pulsars using the Arecibo Observatory. The orbital period of the two neutron stars was found to decrease with time, indicating that they are slowly approaching each other as they expend gravitational radiation, a scenario they had not anticipated but which has since provided tests of gravitation and general relativity (8).

The Bell Telephone Laboratory provided the setting for yet another accidental discovery, involving a horn-reflector antenna that had been used in the first tests of a communication satellite. Arno Penzias and Robert Wilson were measuring the temperatures of all sources of noise in the horn-antenna system to make accurate measurements of the intensity of several extragalactic radio sources. But a persistent, ubiquitous, and unvarying noise

source was detected, contributing an antenna temperature of 3.5 ± 1.0 K, equally strong in all directions, wherever the antenna was pointed, independent of the time of the day and of the year and with no dependence on the location of any known cosmic radio source.

Penzias and Wilson did not know what they had found and avoided any mention of the cosmological implications in their publication. But a group at Princeton University, which was attempting to make a similar measurement at the time, drew attention to the implications in a companion paper. So this particular discovery was not entirely unanticipated. In the late 1940s and early 1950s, George Gamow, Ralph A. Alpher, and Robert C. Herman had previously speculated that the hot, 10^9 K radiation of the early universe would still be around, cooled to about 5 K over the past 14 billion years of expansion. Penzias and Wilson were nevertheless unaware of this work until after their discovery of what is now known as the cosmic microwave background radiation.

So our celestial science seems to be primarily instrument-driven, guided by unanticipated discoveries with unique telescopes and novel detection equipment. With our current knowledge, we can be certain that the observed universe is just a modest fraction of what remains to be discovered. Recent evidence for dark, invisible matter and mysterious dark energy indicate that the main ingredients of the universe remain largely unknown, awaiting future, serendipitous discoveries.

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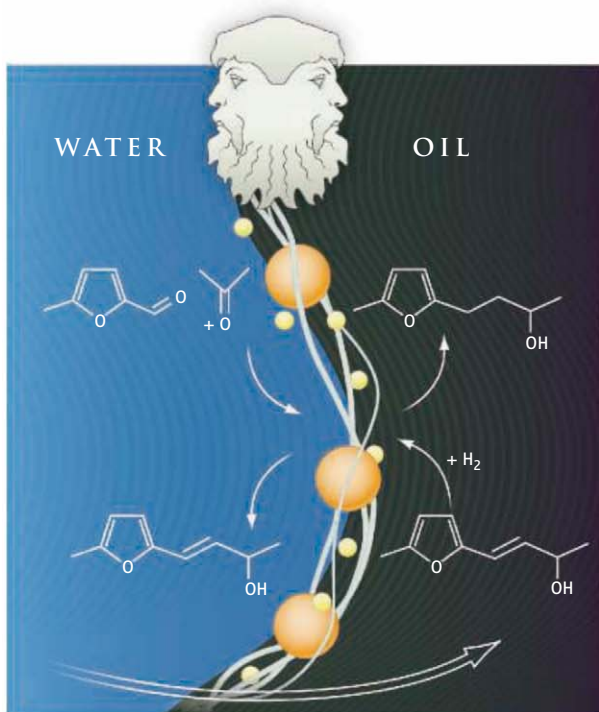
CHEMISTRY

Janus Catalysts Direct Nanoparticle Reactivity

David J. Cole-Hamilton

I'm going to set you a challenge. Go and make a cup of tea. Add milk and sugar, and stir well. Now, please get just the sugar back out for me. Difficult, isn't it? The same problem faces chemists who want to make synthetic products more environmentally friendly. Soluble compounds that are used to speed up desired reactions—homogeneous catalysts—can end up in final products, where they pose a nightmare of a separation problem. Ideally, if these catalysts could be completely recovered, they could be recycled and kept out of the products, in which they could be toxic even at trace levels. One general approach to recovering such catalysts is “phase transfer,” which takes advantage of the different solubility of compounds in water versus organic solvents. On page 68 of this issue, Crossley *et al.* (1) have converted solid nanoparticles that have solubility in both water and oils into catalysts that can operate in both phases. These catalysts can be recovered even from complex mixtures, such as those that result when biomass products are upgraded into fuels.

Phase separation between organic and aqueous phases has been exploited in commercial methods for catalyst recovery (2), but the success of these approaches depends on the particular system. For example, if the reactants have some solubility in water, and the products have less solubility, then separation can be achieved by modifying the catalyst so that it dissolves in water. The catalyst can be recovered at the end of the reaction by simply decanting the oil from the water (3). However, the reaction takes place in the aqueous phase, so if the water solubility of the reactants is too low, the reaction may be unacceptably slow. Some recent approaches have tackled this problem through the use of additives (4, 5) that improve catalyst solubility.



About face for catalysts. Reactions at the interface between organic and aqueous phases (blue and gray areas, respectively) can be catalyzed by different parts of the same nanoparticle. Crossley *et al.* grew carbon nanotubes (shown in white) on metal oxide nanoparticles (shown in orange) that, like the Roman god Janus, present two faces. The oxide surface prefers to be in water and the carbon nanotubes prefer to be in oil, so these particles seek out water-oil interfaces. The addition of palladium (shown in yellow) to these nanoparticles creates catalysts that can work in both phases. In the system depicted here, basic magnesium oxide catalyzes a coupling reaction of 5-methylfurfural and acetone that is useful in biofuel production. The product transfers to the oil phase, where a palladium catalyst attached to the carbon nanotubes catalyzes a subsequent hydrogenation reaction. The nanoparticle catalyst can be separated after reaction via filtration.

Crossley *et al.* have developed nanoparticles that selectively locate at the interface between the aqueous and organic phases. They deposited carbon nanotubes on metal oxide nanoparticles, such as silicon oxide (silica) or magnesium oxide, with diameters of 50 nm or less. The oxides are hydrophilic and attracted to the water, while the carbon nanotubes are hydrophobic and prefer the organic layer (hence they are described as Janus particles, like the two-faced character of mythology). Like a large surfactant molecule, the nanoparticles compromise by sitting at the interface. Unlike surfactants, the nanoparticles are solids that can be separated with methods such as filtration.

Metal oxide nanoparticles decorated with carbon nanotubes can be turned into readily recovered catalysts that function at the interface between oil and water phases.

Achieving high reaction rates depends on creating as much interfacial area between the phases as possible for the catalyst to do its job, which is accomplished by vigorous stirring to create an emulsion. The silica form of these nanoparticles can stabilize either water-in-oil or oil-in-water emulsions (6) and accumulate at high concentrations at the interfaces.

Crossley *et al.* added a catalytically active metal—palladium (Pd)—to these nanoparticles to create catalysts that achieve high reaction rates in biphasic reactions through the combination of high interface concentration and high interfacial surface area. The announcement of rate enhancements alone is an important development, but the authors further advance the field by using their catalysts in reactions to form fuels from bio-oils. Naturally derived molecules are “upgraded” for use as fuels by coupling small molecules together, removing oxygenated groups, or both.

The removal of oxygenated groups illustrates the power of this method. Acid and alcohol groups increase the water solubility of molecules, but if the molecules are coupled together in a way that removes these groups, then the desired product will be more soluble in the organic phase. The catalysts not only accelerate the coupling

reactions, but the biphasic system also allows the desired product to move into the organic phase and leave everything else, including the recoverable catalyst, in the aqueous phase. In systems with a single liquid phase—even ones that use insoluble heterogeneous catalysts on solid supports—all of the products would remain together in solution. Separation would require complex distillation steps that might decompose or rearrange the thermally sensitive products.

Crossley *et al.* also exploit another asymmetry of these Janus particles: The carbon nanotubes grown on silica are more perfect than those grown on magnesium oxide. When they deposit the Pd catalyst in the silica sys-

tem, almost all of it binds on the silica side. Because the catalyst orients itself at the interface with the silica in contact with the water, and the carbon nanotubes in the organic phase, the catalyst is predominantly in contact with the water. Reactants that are water-soluble react more readily in the emulsion than those in the organic phase. Thus, the catalyst will select among reactants in a complex mixture only on the basis of water solubility.

When the Pd catalyst is deposited in the magnesium oxide system, some of it now binds to the carbon nanotubes at defect sites. Hydrogenation reactions can occur in the organic phase, and the magnesium oxide, which is basic, catalyzes coupling reactions in

the aqueous phase. The result is that different reactions can be run in the oil and in the water phases at the same time (see the figure).

One intriguing extension would be to rethink conventional organic synthesis of drug molecules, which depends on several cycles of reaction and separation. A complex molecule could undergo reaction on just one of its functional groups in water. The product could rearrange and become organic-soluble, and then transfer to the other phase, where a different reaction could occur. In this way, a series of reactions could create a desired molecule in one pot, without needing to protect vulnerable functional groups in the reactants. Simple filtration followed by phase separa-

tion and solvent removal would then give the desired product in high yield and containing no catalyst residues—a pharmaceutical chemist's dream come true.

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BIOCHEMISTRY

Some Enzymes Just Need a Space of Their Own

Sebyung Kang and Trevor Douglas

In many ways biology is defined by the idea (and reality) of containers and well-defined barriers. These enable the distinction of self from the rest of the universe, separation of cells from each other, and the definition of organelles within a cell. Although many biological barriers and compartmental boundaries are lipid-based membranes, there is a growing awareness, brought about by some spectacular structural biology, of protein-based compartments that act as isolated environments within the cell. On page 81 of this issue, Tanaka *et al.* (1) add to the growing number of examples of protein-based microcompartments, reporting the structure of a microcompartment that sequesters ethanolamine metabolism in the bacterium *Escherichia coli*. These protein-based containers challenge a long-standing assumption that bacteria and archaea, which lack membrane-enclosed organelles, are devoid of internal compartmentalization.

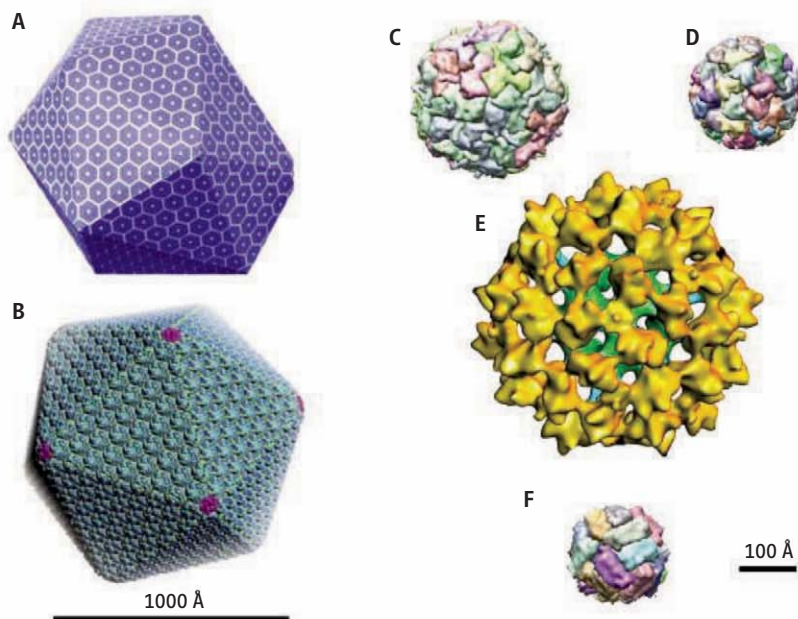
Protein-based compartments often exhibit highly symmetric structures assembled from a limited number of subunit building blocks. These structures are reminiscent of viruses and share properties (2) that make them more than just curiosities—they incorporate catalytic activities and sequester reactions from the cellular environment, and control small-

molecule access across the protein barrier through structural dynamics.

Tanaka *et al.* report the three-dimensional crystal structures of the major shell constituents (EutS, EutL, EutK, EutM) of an ethanolamine utilization (Eut) microcompartment

Protein shells that sequester enzymatic reactions are found in diverse organisms and may provide blueprints for nanoparticle design.

ment, where ethanolamine metabolism is isolated in *E. coli* (see the figure). The Eut microcompartment prevents the release of acetaldehyde into the cytosol, mitigating the potentially toxic effects of excess aldehyde and limiting the loss of carbon by diffusion



Microcompartment architectures. (A) Model of the ethanolamine utilization (Eut) microcompartment. (B) Model of the carboxysome microcompartment. (C) Shell structure of *T. maritima* encapsulin (PDB: 3DKT). (D) Shell structure of *B. subtilis* lumazine synthase (PDB: 1RVV). (E) Three-dimensional reconstruction of bovine pyruvate dehydrogenase complex [derived from (6) with permission from the National Academy of Sciences, USA]. (F) Structure of human ferritin (PDB: 2HFA). Structures in (C), (D), and (F) were generated with the molecular graphics program Chimera, with the indicated PDB files.

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of acetaldehyde across the cell membrane. Although the four Eut shell proteins have similar protein folds, the individual proteins have distinguishing structural features that suit them to specialized architectural and biochemical roles within the microcompartment. EutM and EutS share a conserved α/β core structure that serves as the basic assembly component of the shell. EutL has distinct open and closed crystal forms, which are potentially involved in gated molecular transport through pores in the protein shell. The crystal structure of the carboxyl-terminal domain of EutK suggests that it is a nucleic acid binding protein. Overall, the unique functions of this microcompartment arise from well-defined combinations of its protein components.

Among the examples of protein-based microcompartments found in nature (see the figure) is the carboxysome, which enhances CO₂ fixation inside many photosynthetic and chemoautotrophic bacterial cells by encapsulating the key enzymes ribulose-1,5-bisphosphate carboxylase-oxygenase and carbonic anhydrase. It has a roughly polyhedral proteinaceous outer shell with a diameter of 800 to 1400 Å (3). Encapsulin, isolated from *Thermotoga maritima*, consists of a thin icosahedral shell with a diameter of 240 Å, formed from 60 copies of a single monomer (4). Its interior surface is lined with conserved sites that bind to the carboxyl termini of enzymes involved in oxidative stress response, such as peroxidase. Lumazine synthase, from *Bacillus subtilis*, similarly forms an icosahedral shell and catalyzes the formation of 6,7-dimethyl-8-ribityllumazine in the penultimate step of riboflavin biosynthesis

(5). This icosahedral shell encloses a trimer of a complex called the riboflavin synthase-forming bifunctional enzyme complex (formerly called heavy riboflavin synthase). Similarly, the pyruvate dehydrogenase complex serves as the link between glycolysis and the tricarboxylic acid cycle in many organisms. This complex assembles from multiple copies of three enzymes (a decarboxylase, a dihydrogenase, and a flavoenzyme) to form the icosahedral shell (6). Some protein cage architectures even serve as reaction chambers for inorganic chemistry. Ferritin, for example, is an octahedral protein cage with 24 structurally identical subunits (7). It is found in all domains of life and forms an iron oxide nanoparticle in its cavity as a storage mechanism for maintaining iron homeostasis.

There is a growing interest in mimicking protein compartments for synthetic applications involving the encapsulation and sequestration of catalyst species. To this end, the icosahedral capsid of the cowpea chlorotic mottle virus has been used as a molecular container to encapsulate an individual enzyme of horseradish peroxidase (8), ferritin has been chemically derivatized to incorporate a catalytically active organometallic moiety (7), and a smaller cage constructed from a *Listeria innocua* DNA binding protein has been modified with catalytically active platinum clusters for light-driven H₂ production (9). The advantage offered by these encapsulated catalytic systems lies in the ability to sequester and protect active sites of enzymes, control substrate access, and maintain separation between competing reactions. The potential for artificially incorporating multiple catalytic centers—perhaps part of a

pathway or cascade where high local substrate concentrations can be maintained—has yet to be realized, but the large protein microcompartments, such as the Eut system and large virus-like particles, have the capacity for this level of molecular engineering.

The protein shells of the microcompartments reported by Tanaka *et al.* are not merely static walls or barriers between the interior and exterior environments; they are dynamic skins. Conformational flexibility encoded within protein shells allows selective transportation of components across the barrier and prevents leakage of toxic materials while retaining reaction intermediates. A deep understanding of such fine-tuned dynamics, superimposed on the static architectural features of these microcompartments, may enable the development of bioinspired multifunctional and dynamic nanomaterials as well as fundamental new insights into complex reactions within the cell.

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NEUROSCIENCE

Brain Activity to Rely On?

D. Sam Schwarzkopf¹ and Geraint Rees²

The human brain is a noisy place. The responses of single neurons to sensory stimuli are highly variable. Yet our conscious experience of the environment is stable and consistent. How can a stable conscious representation of the environment arise from noisy individual neuronal responses? Not all activity in the brain reaches consciousness, so one way to address this question is to exam-

ine how neurons respond when we are aware of sensory stimuli relative to when stimuli are unnoticed and invisible. On page 97 of this issue, Schurger *et al.* (1) use functional magnetic resonance imaging (fMRI) to show that in the human brain, visible stimuli that enter awareness elicit spatial patterns of neuronal activity that are more reproducible than for invisible stimuli. This difference may be useful in studying brain function in conditions such as coma or schizophrenia.

fMRI indirectly measures the activity of large populations of neurons simultaneously at many points in the brain, and has provided

The characteristics of neuronal activity that mark whether consciousness arises include how reproducible neuronal response patterns are to a sensory stimulus.

much information about the functional organization of visual processing in the human brain. Even the local spatial pattern of fMRI signals over a few centimeters carries information about underlying neuronal processing (2). Schurger *et al.* have discovered that the consistency of the local spatial patterns of activity in sensory cortex elicited by stimuli changes, depending on whether the stimuli reach awareness or not.

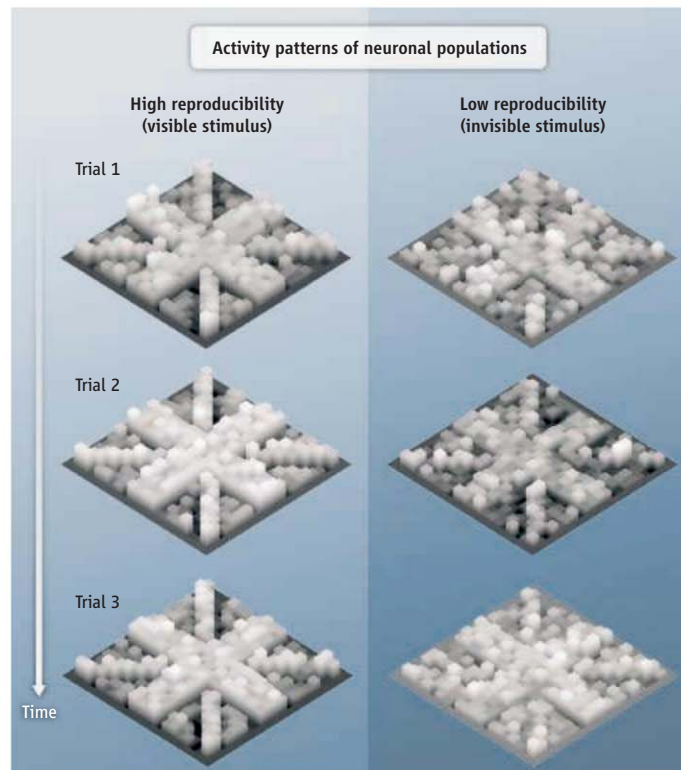
The authors examined brain responses while observers viewed images of faces or houses. Images were presented to each eye simultaneously. If the same image is shown

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to both eyes, it is clearly visible. However, when images of opposite color contrast are presented to each eye, the visual system fuses the two images, and the resultant perception is of a uniformly colored yellow field; thus, the individual images are rendered invisible. Schurger *et al.* examined neuronal populations in the human ventral visual cortex whose activity discriminated whether a face or a house was being presented. They found that the consistency of the spatial patterns of activity in these locations differed depending on whether the stimulus was visible or invisible. If invisible, the patterns were more variable. Thus, invisibility of a stimulus is associated with lower reproducibility of responses, and suggests that reproducibility may be an important difference between conscious and unconscious sensory representations (see the figure).

The main challenge for any study that seeks to compare conscious and unconscious stimulus processing is that changes in brain activity may be simply due to changes in the stimulus (3). Thus, many studies compare physically identical stimuli that differ only in whether they reach awareness. For example, if two deliberately incompatible images are presented to each eye (binocular rivalry), conscious perception alternates spontaneously between each monocular view (4). As stimulation remains constant, changes in conscious contents become dissociated from changes in the physical stimulus. Schurger *et al.* instead used binocular fusion of images presented to both eyes, which allows direct experimental control of whether a stimulus enters awareness. However, in this approach, there is physical variability between stimuli when opposite color contrast images are viewed (making the face or house stimuli invisible), compared to when identical images are viewed (visible stimuli). Many neurons in the primary visual cortex, even if they respond selectively to one eye (5), modulate their responses when dissimilar images are presented to each eye (6). Thus, response variability at this early stage of visual processing might be transmitted on to the higher visual areas explored by Schurger *et al.* In addition, physically different visible and invisible stimuli may elicit differences in involuntary behavior such as the pattern of



Reproducibility. These simulated data illustrate hypothetical activity patterns of neuronal populations in response to three presentations of a stimulus, measured with fMRI in small areas of the human visual cortex. Although each response pattern in the left column carries some noise, the reliability of the pattern across three presentations is apparent. The right column illustrates different response patterns, but of lower reliability. The difference in reliability between the left and right columns is analogous to the difference in response patterns in the human temporal lobe for visual stimuli that either entered awareness and were visible, or remained outside awareness and invisible (1).

very fine eye movements (microsaccades). Such differences will affect the responses of visual neurons and hence the reliability of responses in the visual cortex.

What implications do these findings have for our understanding of how consciousness arises in the brain? Several characteristic signatures of neural activity that are correlated with consciousness have been proposed (7). One suggests that the intensity or the duration of activity in sensory neurons must pass a threshold to reach awareness. Visible images can elicit greater activity in some regions of visual cortex than invisible stimuli (8). Alternatively, consciousness might be correlated with the synchrony of responses in stimulus-selective neuronal assemblies. Schurger *et al.* now add that the reproducibility of responses is a marker of conscious processing.

Reproducibility of response patterns to a sensory stimulus can by definition only be assessed across multiple presentations of that stimulus. But we do not have any difficulty seeing a single stimulus on its own. So reproducibility per se cannot determine whether a

single stimulus enters consciousness or not, but must indicate something about the nature of the underlying mechanisms. One attractive possibility is that recurrent connections between different brain areas reduce the variability of responses for a stimulus that is consciously perceived. Both the strength (9) and direction [feedforward or feedback (10)] of connections may be important determinants of conscious processing. Reduced variability resulting from recurrent processing might align the responses of stimulus-selective neurons across different brain regions. This might explain why Schurger *et al.* were unable to discriminate whether a visible stimulus was a face or a house from the responses of those brain regions that encoded invisible stimuli.

Even in the absence of any stimulation, the brain is active and exhibits highly correlated spontaneous ongoing response patterns (11). Although this activity has sometimes been regarded as noise, it has been suggested that incoming sensory stimulation sculpts the activity and temporal dynamics of these spontaneous networks (12). An alternative explanation for the findings

of Schurger *et al.* is that conscious perception of a stimulus has a direct effect on these spontaneous network dynamics, reducing the variability of their fluctuations. The explanation for the reduced variability of local spatial patterns of responses associated with conscious perception awaits further empirical work.

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RETROSPECTIVE

Rossiter H. Crozier (1943–2009)

Jacobus J. Boomsma and Pekka Pamilo

On 12 November, Ross H. Crozier died unexpectedly from cardiac arrest while at work in his laboratory. He pioneered the use of genetic markers for the study of social insects, made crucial contributions to the theory of social evolution, and was a unique mentor for many who continued in his footsteps.

About 40 years ago, the study of social evolution shifted gears after seminal papers by William D. Hamilton made it clear that the evolution of reproductive altruism could be explained by considering inclusive fitness (the number of gene copies in future generations as they are passed on both via own offspring and the offspring of relatives). This crucial insight allowed Robert L. Trivers to overturn the notion that insect societies are harmonious by default. But these advances only accelerated developments in social insect evolutionary biology after Ross Crozier established the first genetic and statistical techniques to estimate relatedness in groups where pedigrees are unknown. Throughout his career, he bridged the gap between inclusive fitness theory and empirical genetics and mentored two academic generations who applied and elaborated his pioneering initiatives.

Ross Crozier was born in an Australian family living in India during World War II. Already during his school years he was a keen ant observer, an interest that would later shape his academic career. He graduated from the University of Melbourne, where he was influenced by the famous evolutionary cytogeneticist Michael J. D. White, himself a student of J. B. S. Haldane. Crozier went on to do his Ph.D. at Cornell University under the renowned ant biologist William L. Brown. His first scientific papers in the late 1960s mapped chromosomal variation in a large number of ant species.

During his early research, Crozier quickly grasped the potential of using molecular markers to infer family relationships and the importance of inclusive fitness modeling. In the early 1990s, he became the first to take the social insects into the genomics era by sequencing the honeybee mitochondrion. At

this time, sequencing was a highly demanding endeavor because it had to be done manually. In 1993, he published the honeybee mitochondrial genome, with his wife Ching as coauthor (1). It was the second insect mitochondrion to be sequenced, preceded only by *Drosophila*, and it paved the way for the later honeybee genome project.

Soon after receiving his Ph.D. degree in 1969, Crozier became an assistant professor at the University of Georgia. By the time he returned to Australia in 1975, he had initiated the lines of research that were to establish his lasting influence. He showed that the coefficients of relatedness, which are fundamental in Hamilton's inclusive fitness theory, must be formulated differently for ants, bees, and wasps, where males develop from unfertilized eggs. This meant that the entire richness of relatedness differences in the colonies of these insects could be used to test predictions from inclusive fitness theory. He retained his focus on ants, in which differences in relatedness within and among colonies should hold many of the keys for explaining the expression of reproductive conflict from first principles.

Ross Crozier was also the first to realize that Hamilton's concept of animals being able to discriminate between kin, non-kin, and different degree of kin needed genetic underpinning. He formalized the challenges and paradoxes involved, and identified several ways by which kin recognition can evolve. This started a literature in which his original models continue to be cornerstones. He later summarized the state of the field in a monograph with one of us (2). The advances in DNA technology also gave him effective tools to make pioneering contributions to the reconstruction of ant phylogenetic trees and for applying molecular phylogenetics to conservation biology.

Crozier had long-lasting research interests in social insect immunity and the genetics of sex and caste determination, and he kept returning to these topics with new

A geneticist's fascination with ants and other social insects yielded deep insights into the evolution of animal societies.

molecular tools. There are very few areas of social insect evolutionary biology in which he was not involved as a pioneer or later innovator. He aptly demonstrated his scholarship in recent essays on the root of the ant phylogeny (3) and the origin of eusociality (4), in which he opined on recent controversies in his typically modest but insightful manner. In recognition of his contributions to the field, Crozier became the first recipient of the Hamilton Award of the International Union for the Study of Social Insects in 2006.

His characteristic combination of conceptual synthesis and innovative tool development made his laboratories at the University of New South Wales in Sydney, La Trobe University in Melbourne, and James Cook University in Townsville ideal training sites for young researchers and sabbatical havens for senior researchers. Visitors were met with great hospitality and became quickly immersed in the friendly innovative atmosphere that surrounded him.

Crozier's influence has been much more substantial than the list of his coauthors suggests: He created a field and remained an active participant and inspiring tutor through a vast e-mail correspondence across the globe, the messages leaving his home computer often in the evenings or during the weekends. The thoughtful and scholarly constructive way in which Ross Crozier interacted with his peers and students was highly appreciated in the learned societies in which he was active and by the editors of the scientific journals that he served. He will be sadly missed for the new ideas he continued to generate and for his insightful counseling.

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Lipid Rafts As a Membrane-Organizing Principle

Daniel Lingwood and Kai Simons*

Cell membranes display a tremendous complexity of lipids and proteins designed to perform the functions cells require. To coordinate these functions, the membrane is able to laterally segregate its constituents. This capability is based on dynamic liquid-liquid immiscibility and underlies the raft concept of membrane subcompartmentalization. Lipid rafts are fluctuating nanoscale assemblies of sphingolipid, cholesterol, and proteins that can be stabilized to coalesce, forming platforms that function in membrane signaling and trafficking. Here we review the evidence for how this principle combines the potential for sphingolipid-cholesterol self-assembly with protein specificity to selectively focus membrane bioactivity.

The lipid raft hypothesis proposes that the lipid bilayer is not a structurally passive solvent, but that the preferential association between sphingolipids, sterols, and specific proteins bestows cell membranes with lateral segregation potential. The concept has long suffered assessment by indirect means, leading to questions of fact or artifact (1). The resistance of sphingolipid, cholesterol, and a subclass of membrane proteins to cold detergent extraction (2) or mechanical disruption (3) has been widely used as an index for raft association with little or no regard for the artifacts induced by these methods. Though the acquisition of resistance to disruption may point to physiologically relevant biases in lateral composition (4), this disruptive measure tells us little about native membrane organization. Support from light microscopy was also missing because, with the exception of organization into specialized membrane domains such as caveolae or microvilli, putative raft components—specifically glycosylphosphatidylinositol (GPI)-anchored proteins, fluorescent lipid analogs, raft transmembrane (TM) domains, and acylated proteins—often show a homogeneous distribution at the cell surface (5). Moreover, early investigations into submicron membrane organization often yielded conflicting evidence regarding the distribution or motion of these constituents in the living cell (1). Today, however, the advancement of technology has produced compelling data that self-organization of lipids and proteins can induce subcompartmentalization to organize bioactivity of cell membranes.

Origins of the Lipid Raft Concept

Biochemically, it is clear that lipids are sorted within the cell (6). This is particularly notable in polarized epithelia where glycosphingolipids (GSLs) are enriched at the apical surface (7). Lipid rafts were originally proposed as an ex-

planation: Self-associative properties unique to sphingolipid and cholesterol *in vitro* could facilitate selective lateral segregation in the membrane plane and serve as a basis for lipid sorting *in vivo* (7). This proposal for compartmentalization by lipid rafts suggested a nonrandom membrane architecture specifically geared to organize functionality within the bilayer. This function was initially thought to be membrane trafficking; however, rafts could influence organization of any membrane bioactivity (Fig. 1). Here, we highlight advances in technology that point to the existence of raft-based membrane heterogeneity in living cells and discuss the levels of preferential association underlying dynamic domain structure and biological function(s).

Lipid Interactions in Model Membranes

Assembly into two-dimensional liquid crystalline biomembranes is a fascinating property characteristic of lipids. Long thought to be incapable of coherent lateral structure (8), it is now apparent that principles of lipid self-association can also confer organization beyond nonspecific measures of fluidity. An important advance in model-membrane systems was the discovery of phase separation in wholly liquid bilayers (9, 10). It is a cholesterol-dependent lateral segregation, wherein the planarity (molecular flatness) of the rigid sterol ring favors interaction with straighter, stiffer hydrocarbon chains of saturated lipids and disfavors interaction with the more bulky unsaturated lipid species (11). Interaction with cholesterol also forces neighboring hydrocarbon chains into more extended conformations, increasing membrane thickness and promoting segregation further through hydrophobic mismatch (12). In purified lipid systems, the combined effect is a physical segregation in the membrane plane: A thicker, liquid-ordered, Lo phase coexists with a thinner, liquid-disordered, Ld phase (13). Sphingolipids tend to display longer and more saturated hydrocarbon chains, thus potentiating interdigitation between leaflets (14) and favoring interaction with cholesterol. Moreover, unlike glycerophospholi-

pids, the region of chemical linkage between the head group and sphingosine base contains both acceptors and donors of hydrogen bonds, thus increasing associative potential, both with cholesterol and other sphingolipids (11). Other explanations for cholesterol selectivity include the proposed umbrella effect, in which cholesterol hydrophobicity is preferentially shielded by the strongly hydrated head groups of sphingolipid (15) or stoichiometric, but reversible, complex formation between cholesterol and sphingolipid or saturated glycerophospholipid (16).

Immiscible liquid phase coexistence *in vitro* has been suggested as the physical principle underlying rafts *in vivo* (17). Of central importance is the demonstration of selectivity in association between certain lipids. However, phase separation in simple systems at thermodynamic equilibrium *in vitro* cannot be translated into proof for membrane domain formation in living cells (1). Instead, model-membrane work emphasizes the fact that certain lipids exhibit preferential association and provides a framework for understanding how heterogeneity in cell membranes may arise (18). In this respect, the terms Lo and Ld should not be applied to the living cell, as they refer only to the liquid-ordered and liquid-disordered phases of model-membrane systems where parameters relating to translational order (lateral diffusion) and conformational order (trans/gauche ratio in the acyl chains) can be accurately measured (11).

Glimpses of Nano-Assemblies in Living Cells

Currently, lipid rafts are viewed as dynamic nanoscale assemblies enriched in sphingolipid, cholesterol, and GPI-anchored proteins (19) (Fig. 2A). To reach this viewpoint, membrane research has had to contend with the observer's effect, akin to Heisenberg's uncertainty principle: We can change and/or induce heterogeneity in membranes simply by trying to observe it. Initially, this required moving away from detergent extraction as a means to infer native organization. In a first step, detergent-free, chemical cross-linking of GPI-anchored proteins at the plasma membrane suggested that the intrinsic heterogeneity by rafts was present in nanoscale complexes below the optical resolution limit set by the diffraction of light (19). This nanometer-size scale was later supported by viscous drag measures of antibody-bound raft proteins (21) and electron microscopic observation of immunogold-labeled raft antigens (20). Indeed, recent near-field scanning optical microscopy has confirmed a nanoscale bias in the distribution of raft-associating proteins in fixed cells (22). Less perturbing measures of spatial and temporal dynamics in living cells have also provided correlating data. For example, single-particle tracking of colloidal gold-labeled GPI-anchored receptors reveals "stimulation-induced temporary arrest of lateral diffusion," or STALL, in short-lived (~0.5-s) 50-nm areas as a bioactive feature of receptor function (23). Parallel advances in microscopy and spectroscopy have revealed similar heterogeneity

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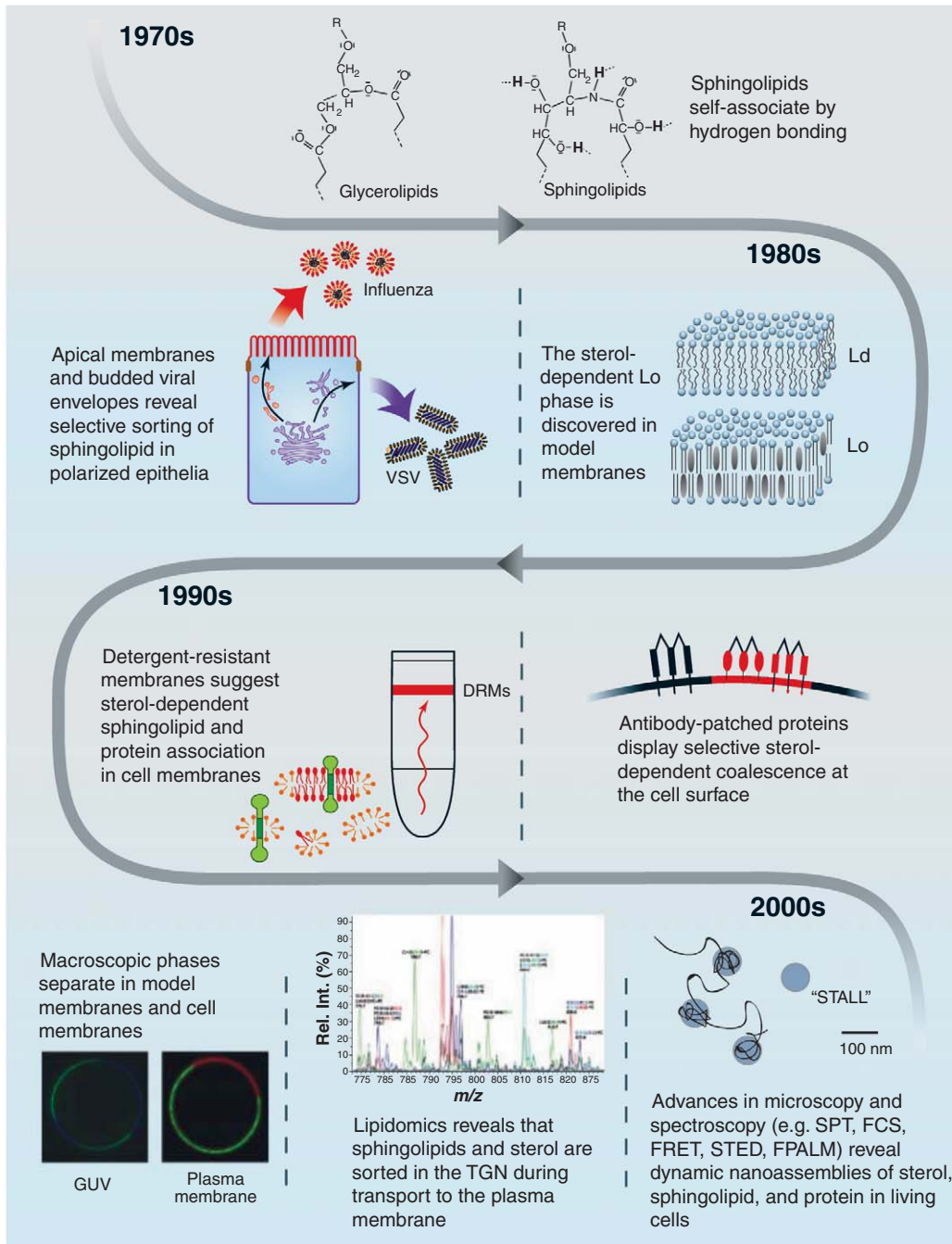


Fig. 1. Evolution of the raft concept for subcompartmentalization in cell membranes. A bold H indicates hydrogen bonding. VSV, vesicular stomatitis virus; DRMs, detergent-resistant membranes; GUV, giant unilamellar vesicle; m/z , mass/charge ratio; SPT, single-particle tracking; FCS, fluorescence correlation spectroscopy; FRET, fluorescence resonance energy transfer; STED, stimulated emission depletion; FPALM, fluorescence photoactivation localization microscopy.

for raft molecules in uncross-linked, “resting” conditions. For GPI-anchored proteins, variable waist fluorescence correlation spectroscopy points to <120-nm assemblages that fluctuate on a sub-second time scale (24). High spatial and temporal resolution fluorescence resonance energy transfer (25) has generated a more conservative size estimate with GPI-anchored receptors residing in more temporally stable clusters of ~10 nm. Assembly formation is always cholesterol-dependent, and, in some cases, an actin requirement has also

been seen (23, 25). However, other techniques have indicated that nanoheterogeneity is actin-independent (26). The situation for TM proteins is not yet clear. However, fluorescence photoactivation localization microscopy has revealed a dynamically clustered nanoscale distribution of hemagglutinin (27), a TM protein previously described as raft-associating (21). The role of the association between cholesterol and sphingolipids in assembly formation has been analyzed recently by stimulated emission depletion mi-

croscopy. This study revealed that, unlike glycerophospholipids, plasma-membrane sphingolipids display transient cholesterol-dependent confinement in areas of <20 nm (28). In this case, differences in diffusion were attributed to differential hydrogen-bonding capacities of glycerol- versus sphingosine-based lipids. However, spin-labeled lipid probes at the cell surface have also revealed heterogeneity in membrane order on an electron spin resonance time scale (29).

Different techniques are yielding a range of values for different molecular constituents in diverse cell types. However, these methods all point to the existence of small, dynamic and selective cholesterol-related heterogeneity in the plasma membranes of living cells. Recent data point to critical behavior as a potential physical basis for the existence of fluctuating nanoscale assemblies in plasma membranes (30).

Functionalization of Nanoscale Heterogeneity

Antibody cross-linking at the cell surface causes raft proteins and lipids to co-patch and exclude non-raft proteins (31). This selectivity in patching is cholesterol-dependent and can be transmitted across the plasma-membrane leaflets (32). The nonrandom coalescence behavior observed in these artifactual cross-linking studies suggests how raft organizing potential may be functionalized to larger, more physiologically relevant temporal and spatial scales (Fig. 2B). A contention of the lipid raft hypothesis is that dynamic nanoscale heterogeneity can be stabilized to coalesce into larger raft domains by specific lipid-lipid, protein-lipid, and protein-protein interactions (20). In this sense, cell membranes would possess an underlying sphingolipid/cholesterol-

based connectivity that can be activated to cluster membrane bioactivity with little energy cost. Indeed, multimerization promotes the sorting of GPI-anchored proteins into sphingolipid/cholesterol-enriched carriers during clathrin-independent endocytosis (33). Along similar lines, clustering of cell surface Gb_3 or GM1 (both GSLs) by their respective ligands Shiga toxin and cholera toxin induces energy-independent tubular invaginations of sphingolipid-biased membrane composition (34, 35). Similar behavior has

also been reported during the multivalent binding of SV40 virus to its GM1 receptor (35). Invagination from the plasma membrane was dependent on having longer receptor hydrocarbon chains, which are common to sphingolipid, and suggests that the effect is mediated by line tension arising between membrane domains of different compositional order (35). Coalescence of dynamic heterogeneity also occurs during signaling, for example, during the formation of B cell receptor (BCR) or T cell receptor (TCR) foci. Antigen binding induces the dynamic association of BCR to its signaling effector Lyn kinase and leads to the formation of an immune synapse. The interaction is dependent on the nature of Lyn lipid anchorage, with membrane order-disrupting bulky hydrocarbon chains preventing association with the BCR (36). During TCR activation, raft components of this receptor complex (e.g., GPI-anchored proteins) become selectively immobilized in nanoscale clusters (37), seeding the accumulation of cholesterol, sphingomyelin, and saturated and long-chain phosphatidyl choline into the synapse, effectively sorting proteins according to their affinity for raft domains (38). Rafts in this “activated” or coalesced condition constitute a more ordered assemblage: a fluid membrane environment in which proteins can be modulated specifically (39), yet that exists separately from the surrounding membrane rich in unsaturated glycerophospholipid. Raft activation is often stabilized or nucleated by scaffolding elements such as cortical actin (40) and may become dominating when the mole fraction of sphingolipids and cholesterol increases, as is the case in the apical membrane of epithelial cells (41).

Phase Separation in Cell Membranes

Despite their selective co-patching with raft markers at the cell surface, raft TM proteins are depleted from the tightly packed Lo phase when reconstituted in model systems (42, 43). Thus, the Lo phase as it exists in simple model membranes is unlikely to be identical to raft-based heterogeneity in plasma membranes that selectively includes TM proteins (44, 45). Giant plasma-membrane vesicles isolated by a chemical membrane blebbing procedure can be cooled to phase separate into Lo- and Ld-like phases (46), and here also, raft TM proteins are typically excluded from the ordered membrane

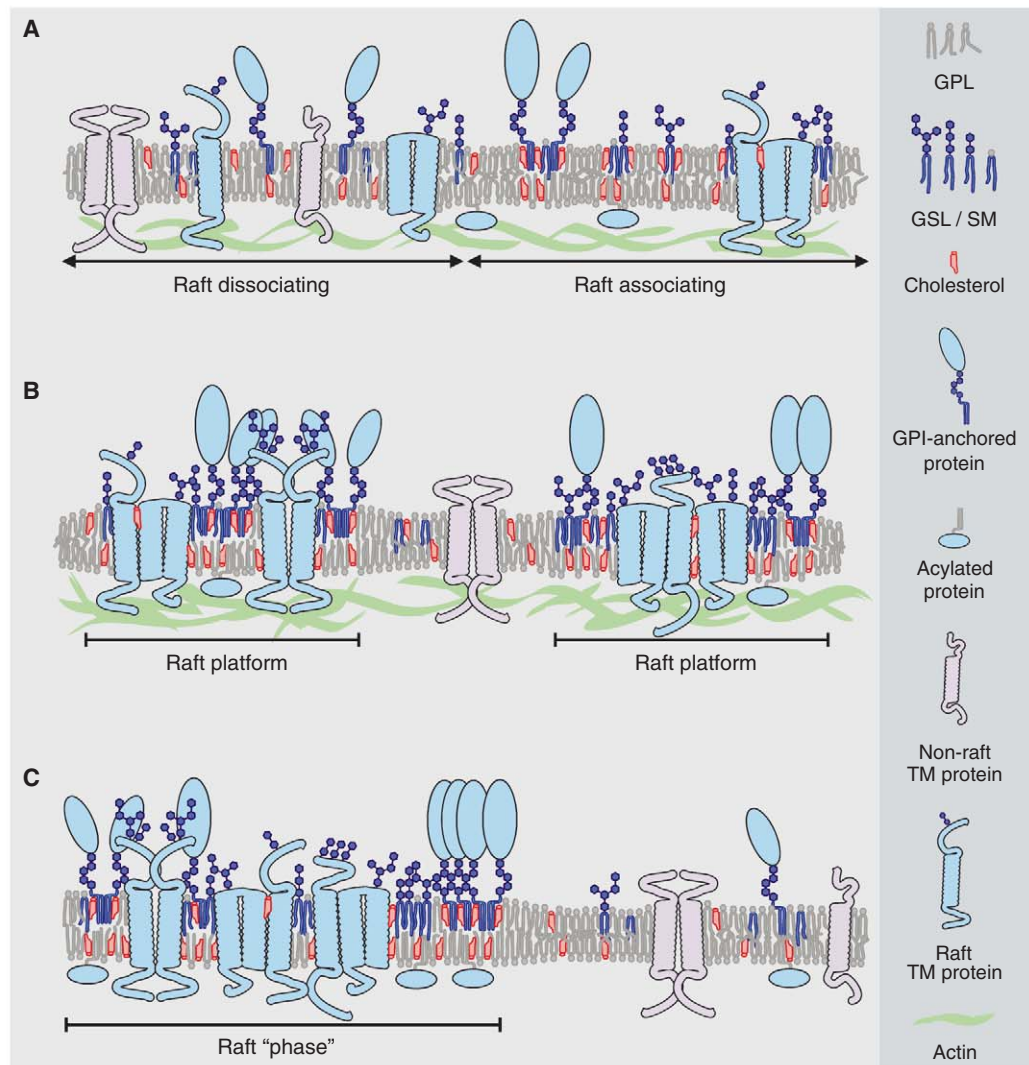


Fig. 2. Hierarchy of raft-based heterogeneity in cell membranes. **(A)** Fluctuating nanoscale assemblies of sterol- and sphingolipid-related biases in lateral composition. This sphingolipid/sterol assemblage potential can be accessed and/or modulated by GPI-anchored proteins, certain TM proteins, acylated cytosolic effectors, and cortical actin. Gray proteins do not possess the chemical or physical specificity to associate with this membrane connectivity and are considered non-raft. GPL, glycerophospholipid; SM, sphingomyelin. **(B)** Nanoscale heterogeneity is functionalized to larger levels by lipid- and/or protein-mediated activation events (e.g., multivalent ligand binding, synapse formation, protein oligomerization) that trigger the coalescence of membrane order-forming lipids with their accompanying selective chemical and physical specificities for protein. This level of lateral sorting can also be buttressed by cortical actin. **(C)** The membrane basis for heterogeneity as revealed by the activation of raft phase coalescence at equilibrium in plasma-membrane spheres. Separated from the influence of cortical actin and in the absence of membrane traffic, multivalent clustering of raft lipids can amplify the functional level to a microscopic membrane phase. Membrane constituents are laterally sorted according to preferences for membrane order and chemical interactions.

phase (47). Remarkably, this phase coexistence indicates that after chemical modification of protein (e.g., formaldehyde cross-linking, thiol treatment), the capacity for physical or lipid-based liquid-liquid phase separation can be manifested by the plasma membrane, despite its compositional complexity. Now the question is, how might phase length-scale separation take place in plasma membranes at physiological temperatures?

Some insight has come from a cell-swelling procedure to separate plasma-membrane spheres

from the influence of cytoskeletal, endocytic, or exocytic processes in a cell line enriched in the raft ganglioside GM1. Pentavalent clustering by cholera toxin resulted in sterol-dependent coalescence of a micron-scale raft “phase” at 37°C, selectively reorganizing the lateral distribution of proteins and lipids according to their predicted affinity for raft domains (44). In this case, selective incorporation of TM proteins was achieved at a lipid-ordering level far below that observed in model-membrane Lo phases (45). Thus, whereas preferential lipid-lipid associations do under-

lie raft clustering, at physiological temperature they do not form a Lo phase when raft proteins become included, and specific lipid-protein interactions must come into play to modify organization (Fig. 2C). Along these lines, a comparison of lipid-packing in vesicles formed from lipids of the plasma membrane versus the plasma membrane itself reveals that lipid domain-forming order is tightly regulated by the presence of protein (48).

Rafts as Entities of Physical and Chemical Specificities

Selective coordination of TM protein organization suggests that cells functionalize lipid order-based sorting by including another specificity, most likely interactions involving proteins (45). Cell membranes are crowded with membrane proteins and their associated biases in regional composition (49). Proteins can specifically organize the distribution of lipids, a property that combines with sphingolipid-cholesterol assemblage potential to produce raft-based membrane heterogeneity.

During vertical distortion of the bilayer, certain lipids of varying chain length are perturbed by the protein surface to different extents via the hydrophobic matching condition. Generally, it is assumed that the hydrophobic membrane-spanning part of the protein is stiff with no appreciable internal flexibility (50). However, by distorting lipids in the vertical direction, it would be possible to counter mismatch. The lipid species best adapted to the matching condition will have an increased probability of being close to the protein-lipid interface (50). Defined as “wetting” (51), the membrane protein surface is proposed to stabilize a sterically favored lipid environment. Electron spin resonance has identified a highly dynamic selection of boundary lipids for a number of proteins (52). However, some membrane proteins retain tightly bound lipids, even in the detergent solution present after purification (53). In such cases, lipids may have defined binding sites, where specific intercalation into protein structure is achieved (54).

Because lipids must vertically complement the rigid hydrophobic surface of the membrane domain of integral membrane proteins, variation in the protein boundary also has direct consequences for the thickness and conformational order of the bilayer (50). In model membranes, long amphiphilic peptides order and thicken the bilayer in the absence of cholesterol, whereas shorter peptides offset the membrane order and thickness induced by the presence of cholesterol (55). It has previously been argued that cholesterol-based increases in membrane thickness influence the subcellular distribution of membrane proteins in accordance with the length of their TM domain (56). Conversely, changes in protein TM length itself have been argued to be the thickness-determining factor (57).

Heterogeneity at the protein boundary is intensified by the fact that most membrane proteins

are oligomeric, acting in specific macromolecular complexes to organize function (49). Superficially, these complexes are a source of steric restrictions and molecular crowding (49), but they can also incorporate specific lipids as integral features of their quaternary structure, thus functionally uniting protein-protein and lipid-protein interactions (54). Lipid incorporation is a function of specific polar-head group interactions and hydrocarbon-chain space-filling functions within the oligomeric complex (54). Many of these oligomeric structures are also formed by strong associations resistant to detergents, with the binding of cholesterol to oligomers of caveolin-1 being a prominent example (58).

In the raft field, we should be asking what it means for proteins to be wetted or, as we define the term in this context, “lubricated” (59), by specific lipids or lipid environments, particularly when it involves constituents that are important components of heterogeneity by rafts. A number

has crafted additional specificity to a lipid-based connectivity, effectively reducing lateral space with minimal energy input. A cholesterol-binding pocket, as well as six palmitate residues, has recently been identified in the crystal structure of the β_2 -adrenergic receptor dimer interface (61). Palmitoylation of some membrane proteins has been shown to enhance association with raft nano-assemblies (62). Perhaps in the context of forming functional protein oligomers, the propensity of palmitate for raft association is augmented by combination with cholesterol. However, whether the β_2 -adrenergic receptor harnesses this lipid to connect with raft-based heterogeneity is not yet clear.

Given the contribution of both physical and chemical specificities to lateral selectivity in the bilayer, lipid rafts are probably functionalized by both lipid-lipid and specific protein-lipid interactions. These lateral associations are governed by both physical and chemical specificity. Lipid-

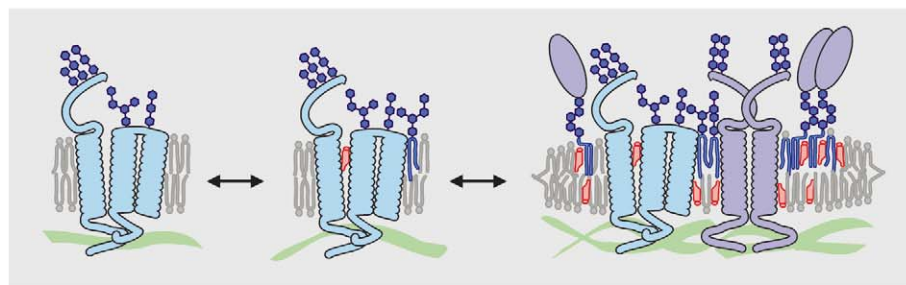


Fig. 3. The lubrication of a raft TM protein by lipid. Membrane proteins bind and/or enrich certain lipids through chemical and physical specificities. These lipids may themselves exhibit sphingolipid/sterol assemblage potential. In this scheme, a TM raft protein (light blue) specifically interacts with sterol and GSL, an interaction that lubricates its inclusion to and the assembly of functionalized (coalesced) raft membrane.

of sphingolipid binding motifs have been described for membrane proteins (60). We propose that specific protein interaction with membrane-ordering “raft lipids” provides a functionalizing connection to the sphingolipid-cholesterol basis for raft assembly (Fig. 3). Interestingly, cholera toxin-cross-linking of GM1 was found to increase the partitioning of the raft enzyme beta-secretase to the Lo phase in giant unilamellar vesicles (43). As pointed out earlier, the Lo phase does not reproduce the modestly ordered, TM protein-inclusive raft structure of cell membranes. Thus, the fact that an undefined specificity for GSLs overcomes this stringent lateral sorting condition suggests that the specific lubrication of protein by lipid is likely to couple proteins to raft-based heterogeneity. Under this scheme, the functionalization of this heterogeneity depends on both lipid physical parameters and specific interactions that may include or even require proteins. For example, the TM protein LAT is an obligate component of raft-based accumulation of membrane-ordering lipids during the formation of the immunological synapse (38). As previously mentioned, membrane proteins work in functional complexes, so it is not surprising that evolution

protein interactions alone cannot describe lipid rafts (63), because these do not account for the preferentially connecting lipid-lipid interactions that have so convincingly been demonstrated in model lipid membranes. Rather, we assert that sphingolipid-cholesterol assemblage potential forms a core raft connectivity that can be precisely modulated by protein specificity. In this view, raft-based membrane heterogeneity couples specific chemistries of association to the physical order preferences of lipids and proteins. Moreover, the assembly of proteins into rafts may be accompanied by conformational changes that modify protein activity.

Rafts Inside the Cell

The propensity to form heterogeneity by rafts is positively correlated with sterol content, which is maximized in the plasma membrane (6, 64), where the actin cortex also plays a central role in influencing or organizing sphingolipid-cholesterol assemblage potential (23, 25). However, for intracellular membranes the situation is less clear. But the emerging field of lipidomics is proving an important tool in evaluating both surface and intracellular membrane heterogeneity (38, 65).

The concentration of sphingolipids and sterols increases along the biosynthetic pathway from the endoplasmic reticulum to the trans-Golgi network (TGN) (65). Recently, the comprehensive lipidome of immunisolated post-Golgi carriers has revealed that order-forming sphingolipids, long and saturated glycerophospholipids, and sterols are selectively enriched in raft protein carriers bound from the TGN to the plasma membrane (66).

Thus, functional raft clustering probably underlies the lateral sorting of cell surface–destined constituents within the TGN, in keeping with the hypothesis of raft “phase” segregation principles as a means to selective carrier formation.

Compositional Evolution of the Cell Membrane

The cellular lipidome is theoretically made up of 9600 species of glycerophospholipids; more than 100,000 species of sphingolipids; thousands of mono-, di-, or triglycerides variants; and numerous fatty-acid and sterol-based structures (67). This amounts to an abundance of composition that might seem geared to dampen collective behavior in the membrane. However, this is not the case. Activating the sphingolipid-cholesterol assemblage potential in thermodynamically equilibrated plasma-membrane spheres leads to demixing of only two “phases” (P) as presently observed (44). The Gibbs phase rule states that the number of de-mixed entities (P) for a system at equilibrium is strictly correlated with the number of chemically independent components (C) by the expression $P = C - F + 2$, where F is the number of independently variable intensive properties (i.e., pressure, temperature, and mole fractions of phase components). Thus, one could venture to postulate that these physical segregation principles have guided the coevolution of both membrane lipid and protein species, such that instead of having the vast P complexity possible from the phase rule, very few different P have survived. This could be explained by the fact that many components of the plasma membrane are not chemically independent, often forming specific complexes to reduce the lateral dimensionality of function. How then can long-range collective behavior arise from a chemically cross-talking plasma membrane? The answer is physicochemical teamwork. Activating the sphingolipid-cholesterol assemblage potential does not involve a purely physical phase transition with defined melting points and the like, but rather the coalescence of raft membrane arises through the functionally relevant cooperation of physical order (from lipid hydrocarbon chains, sterols, and the protein boundary) with specific chemical interactions (between proteins and lipids). In this sense, the cell appears to have designed a membrane composition that manipulates the physically selective behavior of lipids in a chemically specific manner, enabling organized heterogeneity to occur in the living condition.

The introduction of membrane-organizing cholesterol seems to have coincided with the

evolution of multicellular complexity after the oxidation of our atmosphere (68). This correlation may imply that, in the pre-sterol era, other chemical means of reducing lateral dimensionality could have evolved. Interestingly, *Caenorhabditis elegans* does not use sterol as a structural element in its membranes (69). Principles of organized heterogeneity in such organisms are unknown but when revealed will potentially unravel a new chemical toolkit for membrane subcompartmentalization.

Conclusions

Cell membranes are complicated in composition but precise in purpose: to selectively compartmentalize the constituents of life away from environmental lifelessness. Thus, it is not surprising that membranes have innovated a means to laterally organize gatekeepers of this task. In living cells, there is strong evidence for dynamic raft-based membrane heterogeneity at the nanoscale, which can be functionally coalesced to more stable membrane-ordered assemblies. At its core, sphingolipid-cholesterol assemblage potential supplies membranes with a subcompartmentalization propensity that can be accessed or organized by proteinaceous input at little energetic cost. Raft proteins are envisioned as being equipped with a dynamic sterol-sphingolipid-dependent bias in composition at the nanoscale, allowing for the partitioning to and assembly of more stable raft platforms in the functionalized state. During raft activation, protein-lipid interactions are coupled to lipid-order-based sorting, generating heterogeneity serving to functionalize, focus, and coordinate the bioactivity of membrane constituents.

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Masquerade: Camouflage Without Crypsis

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Organisms are under strong selection to avoid detection by predators and to capture prey, and understanding how animals' visual appearances are adapted for these purposes continues to pose interesting questions for evolutionary theory (1). Although the function of crypsis (avoiding detection) (2), aposematism (warning

Selenia dentaria) as prey. Before testing, chicks were divided into nine groups, each containing eight individuals. Birds in all groups received four 2-min trials in which they were placed in the experimental arena individually. The items placed in the experimental arena with them during these trials differed among groups. Three groups encountered

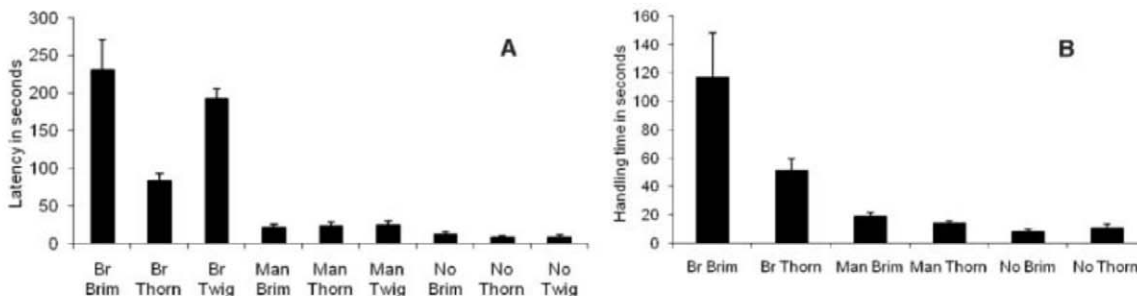


Fig. 1. (A) The time in seconds (mean $\pm$ SE) taken to make a first peck at the test stimulus (Brim indicates brimstone; Thorn, early thorn; and Twig, hawthorn twig). The latency to attack the test stimuli differed among our experimental groups (Kruskal-Wallis test; $\chi^2 = 56.10$, $P < 0.001$, $df = 8$). Specifically, birds trained with branches (Br) took significantly longer to attack the test stimuli than did birds in the control groups trained on manipulated branches (Man) or nothing (No) (Kruskal-Wallis test; $\chi^2 = 47.39$, $P < 0.001$, $df = 1$). Sample size is $N = 8$ in each group. **(B)** The time in seconds (mean $\pm$ SE) that birds handled prey for in the test trial. Handling times (4) differed among our experimental groups (Kruskal-Wallis test; $\chi^2 = 35.34$, $P < 0.001$, $df = 5$). Birds trained with branches took significantly longer to handle caterpillars than did birds in the control groups trained on manipulated branches or nothing (Kruskal-Wallis test; $\chi^2 = 31.16$, $P < 0.001$, $df = 1$). Sample size is $N = 8$ in each group.

coloration) (3), and mimicry (resembling a defended organism) (4) are intensively studied, one aspect of adaptive coloration has been almost completely ignored: masquerade. Masquerading organisms appear to closely resemble inedible and generally inanimate objects such as twigs, leaves, stones, and bird droppings. Individuals using this defensive strategy are assumed to avoid predation or gain access to prey by being misidentified as either inedible objects by their predators or as innocuous objects by their prey. There is currently no empirical evidence to support this theory (5, 6).

Demonstrating that organisms benefit from masquerade is methodologically challenging: It is difficult to determine whether a predator has detected and misidentified an individual (that is, masquerade) (5, 6) or whether it has simply failed to detect the prey item (which would be crypsis) (2). For predators to misidentify a masquerading prey item as the object that it closely resembles, the predator must have previous experience of that object. By manipulating predators' previous experience of the putative model but keeping their exposure to the masquerader the same, it is possible to determine whether predators are misidentifying masquerading prey as their models or simply failing to detect them. We used domestic chicks (*Gallus gallus domesticus*) as predators and putative twig-resembling caterpillars (the Brimstone moth, *Opis-thograpta luteolata*, and the Early thorn moth,

a hawthorn branch complete with leaves (hawthorn *Crataegus* spp. being a common host plant for the two caterpillar species). Three groups encountered a manipulated hawthorn branch that had been bound in purple cotton thread to change its visual appearance without influencing its physical structure or odor. The final three groups experienced an empty arena. The test stimulus differed among groups given the same previous experience: one group received a single Brimstone larvae, another a single Early thorn larvae, and the remaining group received a single hawthorn twig (7).

Birds with prior experience of twigs took longer to attack both species of twig-resembling caterpillars, and handled them more cautiously, compared with birds that had either no experience of twigs or experience only of twigs whose visual appearance had been manipulated by binding them in colored thread (Fig. 1).

Our results show that masquerade functions to promote misidentification of the masquerading organism. The caterpillars in our experiment were treated differently by predators with previous experience of twigs in ways that are likely to lead to antipredatory protection: increased latency to attack and more cautious handling. Most powerfully, this effect occurred at close range, entirely out of context, on a visually contrasting substrate and in an empty arena with no other prey or objects present. We therefore can conclude that masquerade

can provide an entirely additional benefit to crypsis (difficulty in visually detecting prey) (2) and functions even in the absence of crypsis, when the prey is in clear view. Although the role of predator cognition in the evolution of warning coloration is widely studied (3), researchers studying the evolution of other aspects of adaptive coloration have concentrated on understanding how predators' sensory systems have influenced the evolution of prey coloration (2). Our results show that predators' cognitive strategies (recognition and identification), rather than their sensory capabilities, are the selective force driving the evolution of masquerade and raise the possibility that predator cognition may be a more

important selective agent than previously realized.

Masquerade is more widespread than better-studied aspects of adaptive coloration such as aposematism and is found in a large number of species from a wide range of taxa. Plants from the genus *Lithops* look remarkably like stones; stick insects resemble twigs; the Amazon fish *Monocirrhus polyacanthus* is visually almost indistinguishable from leaves, and birds from the family *Nyctibiidae* bear an uncanny likeness to tree stumps.

Therefore, masquerade appears to have evolved on multiple occasions, and its ecological importance requires further investigation in light of our experiments.

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7. Materials and methods are available as supporting material on Science Online.

Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5961/51/DC1
Materials and Methods
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Dendritic Mechanisms Underlying Rapid Synaptic Activation of Fast-Spiking Hippocampal Interneurons

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Fast-spiking, parvalbumin-expressing basket cells (BCs) are important for feedforward and feedback inhibition. During network activity, BCs respond with short latency and high temporal precision. It is thought that the specific properties of input synapses are responsible for rapid recruitment. However, a potential contribution of active dendritic conductances has not been addressed. We combined confocal imaging and patch-clamp techniques to obtain simultaneous somatodendritic recordings from BCs. Action potentials were initiated in the BC axon and backpropagated into the dendrites with reduced amplitude and little activity dependence. These properties were explained by a high K^+ to Na^+ conductance ratio in BC dendrites. Computational analysis indicated that dendritic K^+ channels convey unique integration properties to BCs, leading to the rapid and temporally precise activation by excitatory inputs.

Fast-spiking, parvalbumin-expressing, γ -aminobutyric acid (GABA)-releasing (GABAergic) interneurons (BCs) play a

key role in the function of neuronal networks. These neurons set a narrow time window for temporal summation in principal neurons by fast

feedforward and feedback inhibition, contribute to the generation of network oscillations, and are thought to be involved in higher brain function and dysfunction (1–7). After stimulation of excitatory input synapses in vitro, BCs respond with remarkable speed, exquisite temporal precision, and preferential activity in the onset phase of a stimulus train (8, 9). Similarly, during network activity in vivo, such as sharp-wave ripple or theta rhythms, BCs are activated by input from principal neurons with short latency and minimal jitter (10–13). The mechanisms underlying this rapid and precise activation are unclear. It is generally thought that synaptic factors, such as the time course of the postsynaptic conductance and the extent of depression or facilitation, play an im-

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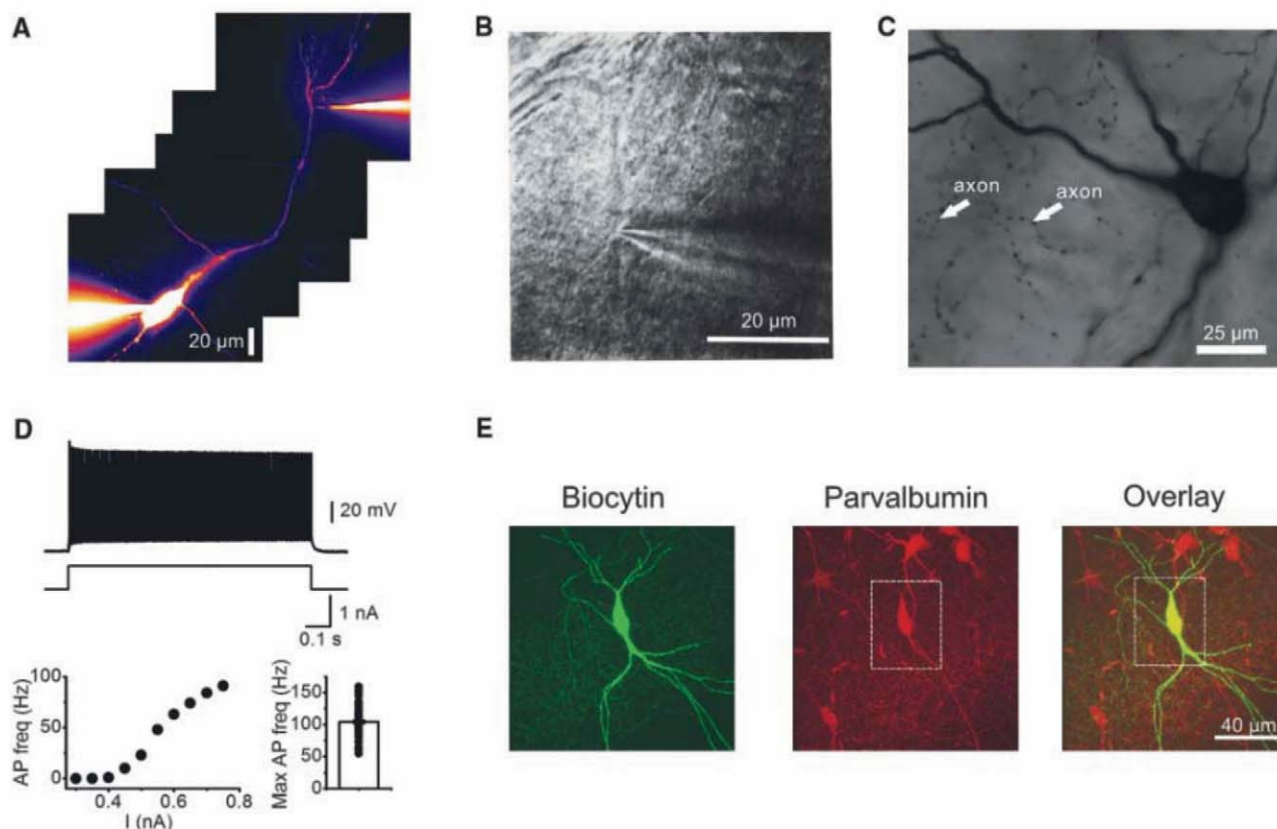


Fig. 1. Confocally targeted recording from BC dendrites. (A) Confocal image (pseudocolor representation) of a BC in the dentate gyrus filled with Alexa Fluor 488 taken during the experiment. (B) Infrared differential interference contrast videoimage of the apical dendrite of the same cell as in (A). (C) Light micrograph of a BC filled with biocytin during recording and labeled by using 3,3'-diaminobenzidine. 10-μm stack projection. Arrows indicate the axonal arbor, forming "baskets" around granule cell somata. (D) Train of APs

evoked by a 1-s, 0.75-nA current pulse applied at the soma (top) and AP frequency (f) – current (I) relation (bottom). Same cell as in (C). Bottom right graph shows mean maximal AP frequency (bar) and data from individual cells (points). (E) (Left) Confocal micrograph of a BC filled with biocytin and stained with fluorescein isothiocyanate (FITC)-conjugated avidin; (center) parvalbumin immunoreactivity of the same BC; (right) overlay. 40-μm stack projection.

portant role (8, 9, 14–16). Alternatively or additionally, the electrical properties of interneuron dendrites may contribute. Whereas the dendrites of pyramidal neurons were extensively characterized (17–22), those of fast-spiking, parvalbumin-expressing BCs have not been directly examined. However, Ca^{2+} imaging experiments suggest that Na^+ , K^+ , and Ca^{2+} channels may be present in the dendrites of neocortical fast-spiking interneurons (23).

To study the dendrites of BCs directly, we used confocally targeted patch-clamp recording in hippocampal slices (Fig. 1) (20). After filling BCs in the dentate gyrus with Alexa Fluor 488 via somatic recording, we traced dendrites into molecular layer or hilus by using confocal imaging (Fig. 1A) and obtained dendritic recordings by using correlated infrared-differential interference contrast videomicroscopy (Fig. 1B). BCs were identified on the basis of the location of the axon in the granule cell layer (24) (Fig. 1C), the fast-spiking AP phenotype (25) (Fig. 1D; mean maximal frequency 104.2 ± 2.2 Hz; $n = 98$ dual recordings), and the immunoreactivity to the Ca^{2+} -binding protein parvalbumin tested in a subset of neurons (Fig. 1E; 23 of 25 cells). Detailed anal-

ysis of the axonal arbor revealed that our sample was mainly composed of classical basket cells with tangential collaterals (78 of 83 recovered cells) but also included a subpopulation of cells with radial collaterals, suggestive of axo-axonic cells (5 of 83 cells) (fig. S3) (26).

APs propagate into BC dendrites with attenuated amplitude. We made simultaneous recordings from somata and apical dendrites of dentate gyrus BCs at distances up to 300 μm from the soma, close to the physical dendritic length (Fig. 2). Current injection at both the soma and the apical dendrite evoked high-frequency trains of APs (Fig. 2A). Although the AP frequency was identical at the soma and the dendrite, single APs at the two locations differed substantially (Fig. 2B). First, APs in the apical dendrite showed markedly attenuated amplitude in comparison with somatic APs. For the first AP in the train, the peak amplitude measured from threshold was 80.8 ± 2.0 mV at the soma and 23.4 ± 2.1 mV at the dendrite (at distances >100 μm from soma; $P < 0.01$; Fig. 2, B and D). Second, the maximal rate of rise declined as a function of distance (444.4 ± 29.5 mV ms^{-1} at the soma versus 81.3 ± 10.8 mV ms^{-1} at the dendrite at

>100 μm ; $P < 0.01$; Fig. 2, B and E). Lastly, the duration at half-maximal amplitude was slightly prolonged (0.50 ± 0.02 ms at the soma versus 0.84 ± 0.08 ms at the dendrite at >100 μm ; $P < 0.01$; Fig. 2, B and F).

To test whether dendritic AP propagation was activity-dependent, we compared the properties of the first and the last AP at distal dendrites in the high-frequency train (Fig. 2, B and C). The amplitude of the dendritic AP at the end of the train was similar to that at the onset (22.0 ± 1.8 mV versus 23.4 ± 2.1 mV; $P > 0.5$ for distal recordings >100 μm). Likewise, the maximal rate of rise and the duration at half-maximal amplitude were comparable between the first and the last AP (Fig. 2, D to F). To examine whether the properties of the dendritic AP depended on the site of current injection, we compared APs evoked by somatic and dendritic current injection in the same cell (Fig. 2, B to C). Dendritic APs evoked by somatic and dendritic current injection had similar amplitudes (22.7 ± 2.8 mV versus 24.8 ± 3.4 mV for the first AP; $P > 0.4$ for distal recordings). Likewise, the maximal rate of rise and the duration at half-maximal amplitude were independent of the site of current injection (Fig. 2, D to F).

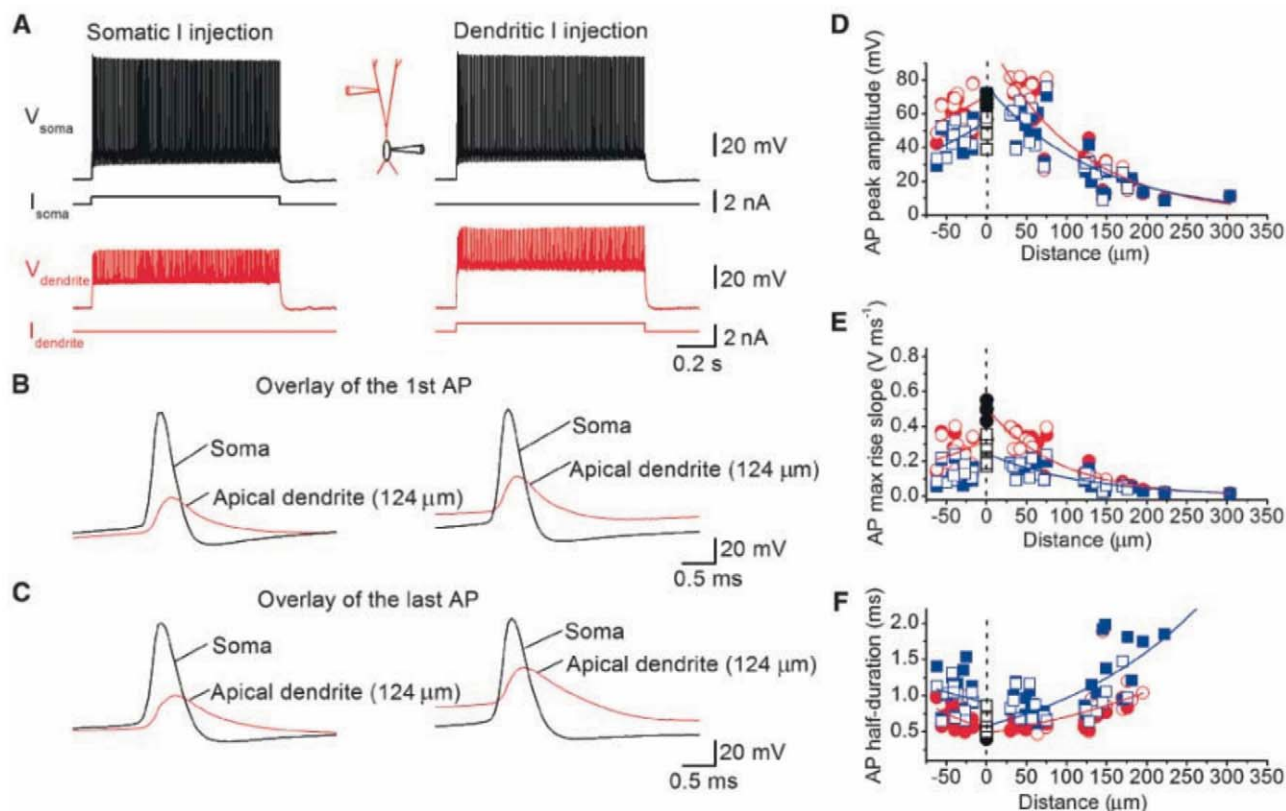


Fig. 2. APs in BC dendrites show marked amplitude attenuation, moderate broadening, and little activity dependence. (A) Train of APs evoked by somatic (left) and dendritic (right) current injection. Black traces, somatic voltage and corresponding current; red traces, dendritic voltage and corresponding current. (B and C) First AP (B) and last AP (C) in the 1-s train shown at expanded time scale. Dendritic recording site on apical dendrite 124 μm from soma. (D to F) Summary plot of AP peak amplitude measured from threshold (D), maximal slope of the rise (E), and duration at half-maximal amplitude (F) plotted against distance (positive distance,

apical dendrite; negative distance, basal dendrite; both measured from the center of the soma). Data from 42 simultaneous somatodendritic and 8 somatic recordings (distance 0). Recording temperature $\sim 23^\circ\text{C}$. Solid symbols, somatic current injection; open symbols, dendritic current injection. Red, first AP; blue, last AP in a 1-s train. For the half-duration of the first AP, only 39 out of 42 recordings could be analyzed in which voltage crossed the half-maximal amplitude level during repolarization. Lines represent exponential functions fit to the data points. For length constant values, see table S1.

Analysis of APs at near-physiological temperature gave comparable results (fig. S1). Furthermore, APs recorded in basal dendrites [receiving granule cell input (14)] were similar to those in apical dendrites (receiving mossy cell and entorhinal cortex input) (fig. S2). Lastly, similar results were obtained for neurons with radial axon collaterals, representing putative axo-axonic cells (fig. S3).

APs are initiated in the axon. To determine the site of AP initiation, we measured latency differences of APs at different subcellular locations (Fig. 3). In the majority of apical dendritic recordings (19 of 28 BCs), somatic APs preceded dendritic APs during long current pulses, independently of the site of current injection (Fig. 3, A and B). When measured at the half-maximal amplitude, the somatic AP preceded the dendritic AP by 0.21 ± 0.05 ms (14 apical dendritic recordings at distances of >100 μm). Likewise, in the majority of basal dendritic recordings (12 of 14 BCs), the somatic AP preceded the dendritic AP (dendritic recordings 17 to 62 μm from the soma).

In pyramidal neurons, brief high-intensity dendritic current pulses or distributed activation of excitatory synapses trigger dendritic spikes more effectively than long current pulses (27–29). We therefore tested these stimulation paradigms in BCs. However, 0.5-ms current pulses applied to the dendrite (137 ± 17 μm) failed to initiate dendritic spikes in six out of six BCs and even failed to trigger somatic APs in four out of six recordings, despite the injection of large current amplitudes of up to 3.5 nA (Fig. 3C). Failure of AP initiation was presumably related to the marked rectification of the voltage-current relation, showing that it becomes increasingly difficult to depolarize the dendritic membrane potential beyond -30 mV (Fig. 3C). Activation of distal synapses by stimulation of axons of the lateral perforant path initiated APs (Fig. 3D). However, as with the other stimulation paradigms tested, the somatic AP constantly preceded the dendritic AP. Similarly, stimulation of two synaptic inputs with stimulation electrodes ~ 30 μm apart initiated APs in which the somatic consistently preceded the dendritic response (four BCs; fig. S4).

In a subset of BCs, either the dendritic APs preceded the somatic APs (10 of 42 BCs) or dendritic and somatic APs occurred simultaneously (1 of 42 BCs; Fig. 3, E and F). This could be due to either dendritic AP initiation (27–29) or dendritic origin of the axon (30–32). We therefore performed a correlated analysis of the morphological properties of BCs by using post-hoc biocytin labeling. In all cases in which the AP occurred first in the dendrite, the axon originated from the dendrite and was closer to the dendritic than the somatic recording electrode (Fig. 3G). To test whether the results from all morphologically recovered BCs were quantitatively consistent with axonal AP initiation, we plotted AP latency against the difference of distances of the recording locations from the axon origin (Fig. 3H). The linear regression line intersected the abscissa near 0, indicating AP initiation in the axon (correlation coefficient $r = 0.90$; $P < 0.001$).

High K^+ to Na^+ conductance ratio in BC dendrites. Both the robust axonal AP initiation and the marked dendritic AP attenuation could suggest that BC dendrites behave as passive cables. We therefore determined the density of voltage-gated Na^+ and K^+ channels in outside-out patches isolated at various locations (Fig. 4). In somatic outside-out patches obtained with Cs^+ -internal solution, voltage pulses from -120 mV to 0 mV evoked inward currents in the majority of

patches (Fig. 4A). These currents showed fast activation and inactivation and were blocked by 1 μM tetrodotoxin in the external solution, demonstrating that they were mediated by voltage-gated Na^+ channels (33) (Fig. 4B). In contrast, in dendritic outside-out patches, the amplitude of inward currents was substantially smaller (Fig. 4, A and C). Quantitative analysis revealed that the Na^+ current density was 13.3 ± 2.1 pA μm^{-2} at the soma but steeply declined as a

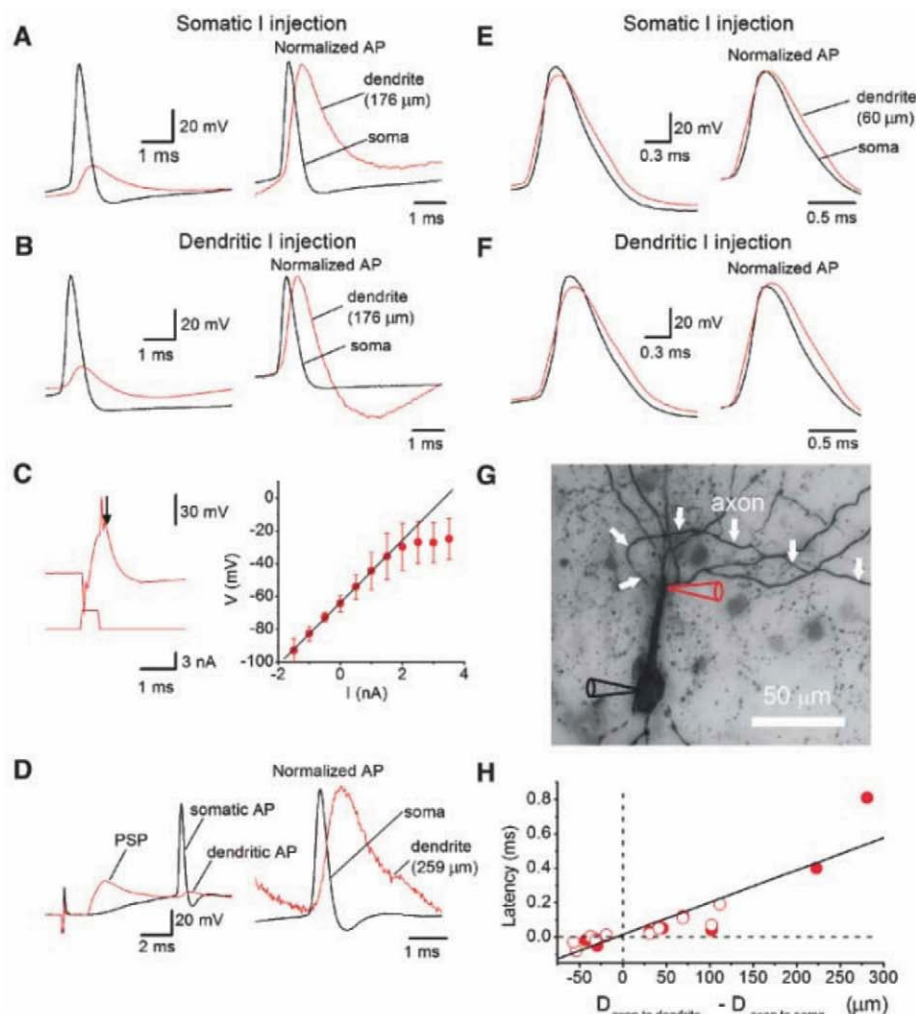


Fig. 3. The axon is the exclusive site of AP initiation in BCs under a variety of conditions. **(A and B)** Recording from a BC in which the somatic preceded the dendritic AP (31 of 42 BCs). First somatic (black) and dendritic (red) APs evoked by 1-s somatic (A) and dendritic (B) current pulses. Left graphs, somatic and dendritic APs at absolute voltage scale; right graphs, APs normalized to same peak amplitude. Dendritic recording site on apical dendrite 176 μm from soma. **(C)** Responses to brief depolarizing current injection in the dendrite. (Left) Dendritic responses and (right) voltage-current relation for the voltage immediately after the pulse (arrow) in a subset of BCs in which no somatic spikes could be elicited (four out of six cells). Line represents linear regression of data points for voltages ≤ -30 mV. **(D)** Somatic (black) and dendritic (red) APs initiated by evoked postsynaptic potentials (PSPs). Axons of the lateral perforant path were stimulated in the outer molecular layer. Left traces, somatic and dendritic APs at absolute voltage scale; right traces, APs normalized to same peak amplitude. **(E and F)** Recording from a BC in which the dendritic preceded the somatic AP (10 of 42 BCs). First somatic (black) and dendritic (red) APs evoked by 1-s somatic (E) and dendritic current pulses (F). **(G)** Correlated light microscopic morphological analysis of the same cell shown in (E and F). The axon (arrows) originated near the dendritic recording site. **(H)** Plot of latency between somatic and dendritic APs against the corresponding difference of distances, using the axon origin as reference point. Solid circles, somatic current injection; open circles, dendritic current injection. Line represents the result of linear regression; average dendritic AP propagation velocity was 0.53 m s^{-1} . Recording temperature $\sim 23^\circ\text{C}$ except for synaptic experiments in (D) ($\sim 31^\circ\text{C}$).

function of distance, with estimated length constants of 87 μm in apical dendrites and 25 μm in basal dendrites (Fig. 4D and table S1).

Expression of Kv3-type K^+ channels is a hallmark property of fast-spiking GABAergic interneurons (34, 35). We therefore explored the possible presence of Kv3 or other types of K^+ channels (19, 36) in BC dendrites. In somatic outside-out patches isolated with K^+ -internal solution, voltage pulses from -120 mV to 70 mV evoked large voltage-dependent outward currents (Fig. 4E). Quantitative analysis revealed a K^+ current density of 91.5 ± 21.1 pA μm^{-2} at the soma. In apical dendrites, the K^+ current density decayed moder-

ately as a function of distance, with an estimated length constant of 763 μm (Fig. 4F). In contrast, in basal dendrites, the decay was steeper, with a length constant of 57 μm .

To determine the molecular identity of the K^+ channels expressed in BC dendrites, we measured functional parameters that discriminate among K^+ channel subtypes (25, 34, 35) (fig. S5). Dendritic K^+ channels in BCs showed several characteristic properties. First, they had a high activation threshold of ~ 40 mV (fig. S5, a and b). Second, their activation time course was fast and highly voltage-dependent (fig. S5c). Third, they were blocked by low concentrations of extra-

cellular tetraethylammonium (TEA), with only minimal distance dependence (fig. S5, d and e). Application of 1 mM TEA reduced the K^+ current amplitude in dendritic patches to $62.7 \pm 5.7\%$, similar to somatic patches ($P > 0.05$; fig. S5e), whereas 200 nM α -dendrotoxin (α -DTX) had almost no effect (amplitude $97 \pm 0.6\%$ of control; $n = 3$). Lastly, K^+ channels showed only minimal inactivation, again with little distance dependence (fig. S5f). Because only Kv3 channels combine the properties of high activation threshold, rapid activation, high TEA sensitivity, and insensitivity to α -DTX, our results indicate that Kv3-type channels prevail in BC dendrites, consistent with immunocytochemical data [(34, but see (23))].

Dendritic K^+ channels shape excitatory postsynaptic potential (EPSP) time course and coincidence detection in BCs. One implication of our results is that dendritic K^+ channels may be efficiently activated by excitatory synaptic input (8, 9, 14–16). We therefore injected artificial excitatory postsynaptic-current-like (EPSC-like) waveforms (aEPSCs) into the dendrite (231 ± 12 μm) while recording the corresponding artificial EPSPs (aEPSPs) at the soma (Fig. 5). The parameters of the aEPSCs were chosen to mimic the amplitude and time course of unitary EPSCs in BCs (14). Bath application of 5 mM TEA reversibly prolonged the half-duration of single aEPSPs from 12.5 ± 0.7 ms to 14.8 ± 0.9 ms ($P < 0.01$; Fig. 5A). Because TEA had no significant effect on the membrane time constant ($\tau = 10.8 \pm 0.8$ ms in control versus 12.0 ± 1.8 ms in TEA; $P > 0.2$), these results indicate that voltage-gated K^+ channels accelerate the decay of EPSPs (37).

To corroborate that the K^+ channels activated by EPSPs were located dendritically, we simulated EPSPs in a previously established passive BC cable model (Fig. 5B) (38) with and without dendritic voltage-gated K^+ channels. Insertion of K^+ channels into the apical dendrites near the activated synapses accelerated EPSP kinetics (Fig. 5, C and D), reproducing the experimental observations (Fig. 5A). In contrast, insertion of K^+ channels into the basal dendrites remote from the synapses had only minimal effects on EPSP kinetics.

Activation of BCs requires the coincident activation of multiple excitatory synaptic inputs (8, 14). To test how the K^+ channel-mediated acceleration of the EPSP decay time course affects coincidence detection in BCs, we simulated pairs of EPSPs separated by variable time intervals (Fig. 5, E and F). If the same synapse was activated repetitively at variable time intervals, dendritic K^+ channels reduced the extent and shortened the time window of temporal summation (Fig. 5E). In contrast, if two spatially separated synapses were activated at different times, the maximal extent of summation was unchanged, whereas the summation window was shortened by dendritic K^+ channels (Fig. 5F). Thus, dendritic K^+ channels enable BCs to selectively detect nearly coincident, spatially separated activity.

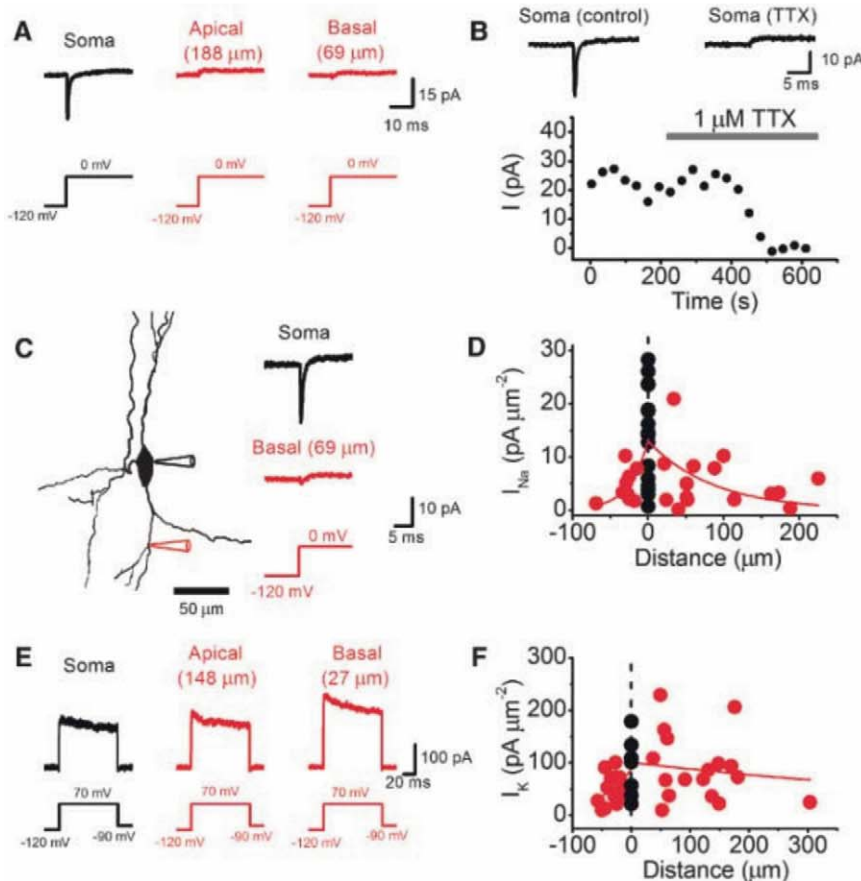


Fig. 4. High K^+ to Na^+ conductance density ratio in BC dendrites. (A) Na^+ currents evoked in outside-out patches from soma, apical dendrite, and basal dendrite. Test pulse potential was 0 mV. Na^+ currents were recorded with Cs^+ -internal solution. (B) Tetrodotoxin (TTX) sensitivity of Na^+ channels. Top graphs, Na^+ currents in control conditions and in the presence of 1 μM TTX (somatic outside-out patch). Bottom, plot of Na^+ peak current against time during TTX application (horizontal bar); each point represents the mean of 10 consecutive measurements. (C) Difference between somatic and dendritic Na^+ channel density in the same cell. (Left) Location of somatic and dendritic recording pipette, superimposed with morphological reconstruction of the somatodendritic domain of the BC. (Right) Na^+ currents recorded in outside-out patches isolated from these locations by using two patch pipettes pulled from same glass capillary. (D) Na^+ current density as a function of distance from the soma. Data from 17 somatic (black), 15 apical dendritic (red, positive distances), and 9 basal dendritic (red, negative distances) outside-out patches. (E) K^+ currents evoked in outside-out patches from soma, apical dendrites, and basal dendrites. Test pulse potential was 70 mV. K^+ currents were recorded with K^+ -internal solution. (F) K^+ current density as a function of distance from the soma. Data from 7 somatic (black), 17 apical dendritic (red, positive distances), and 14 basal dendritic (red, negative distances) outside-out patches. Red lines represent exponential functions fit to the data points. For length constant values, see table S1. Na^+ currents are the average of 38 to 50 sweeps; K^+ currents are either single traces or the average of three sweeps. Leakage and capacitive currents were digitally subtracted.

Dendritic K⁺ channels control EPSP-AP coupling and AP phenotype of BCs. To test how dendritic K⁺ channels influence EPSP-AP coupling, we inserted Na⁺ and K⁺ channels in densities consistent with our experimental results and compared scenarios with passive and active dendrites (Fig. 6). In the BC model with passive dendrites, APs evoked by both brief current pulses and synaptic

activation were followed by a marked afterdepolarization. In contrast, in the model with active dendrites, the afterdepolarization was largely suppressed (Fig. 6B). Analysis of the underlying mechanisms revealed that the activation of dendritic K⁺ channels, which was particularly efficient for APs evoked by EPSPs, reduced the dendrosomatic current flow after APs (Fig. 6B).

Correlated with this inhibition of the afterdepolarization, dendritic K⁺ channels changed the input-output characteristics of BCs. In the model with passive dendrites, strong synaptic stimuli (activation of >25 synapses) triggered bursts of APs. In contrast, in the model with active dendrites, single APs were generated over a wide range of synaptic stimulus intensities (Fig. 6C). Furthermore, with a repetitive synaptic stimulation paradigm the model with passive dendrites showed a greatly reduced AP initiation threshold for the second synaptic stimulus, whereas the model with active dendrites had an almost constant AP initiation threshold (Fig. 6D). Thus, dendritic K⁺ channels generate strength- and timing-independent activation properties of BCs.

Lastly, dendritic K⁺ channels contribute to the classical fast-spiking AP phenotype of BCs (25). In the model with passive dendrites, long depolarizing current pulses evoked trains of APs with markedly declining amplitude, and APs were occasionally initiated after the termination of the pulse (Fig. 6, E and F). In contrast, in the model with active dendrites, the AP amplitude was relatively constant during the train.

Discussion. The dendrites of BCs differ from those of pyramidal neurons in several ways. First, in BCs, the axon is the invariant AP initiation site. In contrast, in pyramidal neurons APs are preferentially triggered in the axon (17, 18) but can be also initiated in dendrites under specific conditions (27–29). Second, in BCs, APs backpropagate into dendrites with marked amplitude attenuation, but little AP broadening and minimal activity dependence. In contrast, in pyramidal neurons backpropagation is less decremental and more activity-dependent (17, 18). Third, BC dendrites are endowed with a low Na⁺ channel density but a high Kv3 channel density, whereas pyramidal neuron dendrites show a high density of both Na⁺ channels and A-type (Kv4) K⁺ channels (19, 36). Thus, Kv3 channels are expressed in dendrites, somata, and axons of fast-spiking interneurons (35, 39). Lastly, K⁺ channels in BC dendrites shorten the EPSP time course, whereas Na⁺ channels in pyramidal neuron dendrites boost the amplitude and prolong EPSPs (37, 40).

Dendritic K⁺ channels shape the electrical properties of fast-spiking GABAergic interneurons at the level of input, EPSP-AP conversion, and output. At the input level, dendritic K⁺ channels accelerate the decay time course of unitary EPSPs, reduce the time window for temporal summation, and help BCs to detect the synchronous activity of converging, spatially separated excitatory inputs. Dendritic location puts K⁺ channels into a strategic position to be efficiently activated by EPSPs and enables them to implement synapse-specific processing rules. At the level of EPSP-AP conversion, dendritic K⁺ channels ensure precise 1:1 coupling between EPSP and AP, suppressing the generation of AP bursts by large synaptic inputs. Furthermore, dendritic K⁺ channels ensure a constant AP ini-

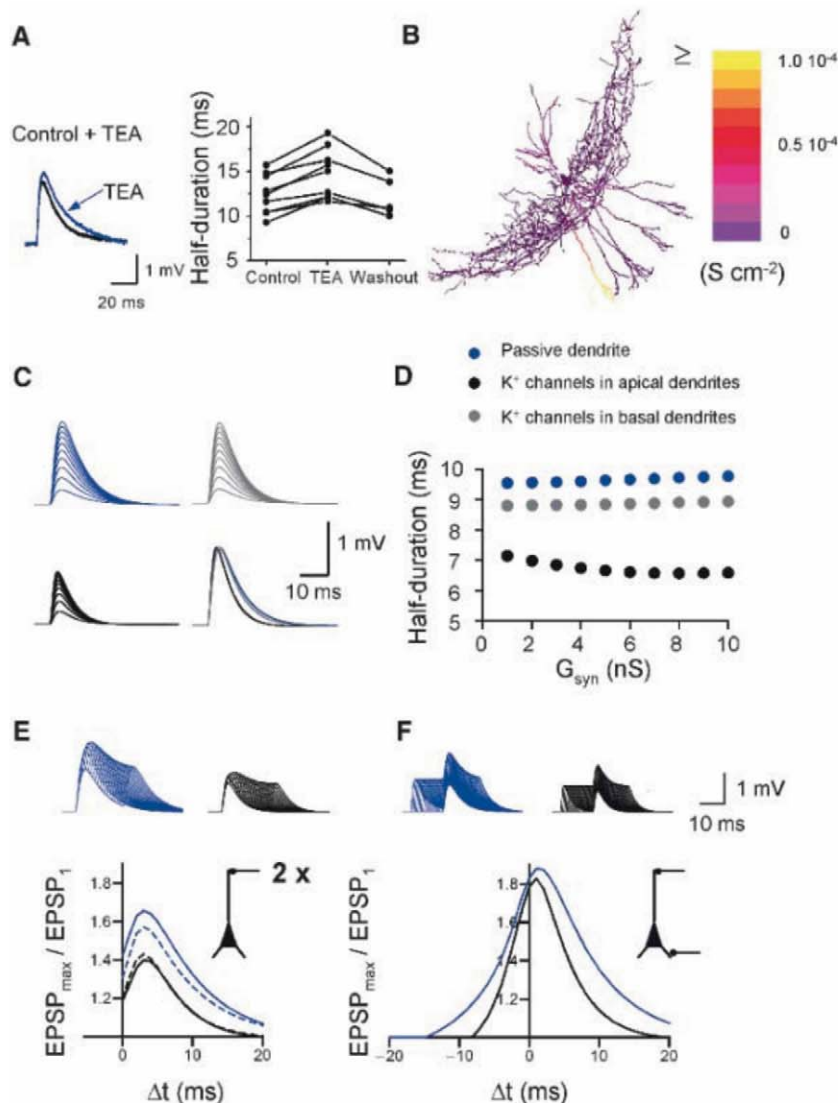


Fig. 5. Dendritic K⁺ channels shape EPSPs and coincidence detection properties of BCs. (A) (Left) Artificial EPSPs (aEPSPs) evoked by injection of an EPSC-like current into the dendrite and recorded at the soma in control conditions (black) and in the presence of 5 mM TEA in the extracellular solution (blue). Peak amplitude of the aEPSP was 0.5 nA. (Right) Summary graph of half-duration of the aEPSP. Data from the same experiment are connected by lines. Peak amplitude of aEPSP was 0.5–3 nA; 10 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2 μ M SR95531 were added in four out of nine experiments. (B) Simulation of K⁺ channel activation in a BC 2 ms after onset of an EPSP in the distal apical dendrite. Color code (right) shows density of activated K⁺ conductance. (C) Dendritic K⁺ channels lead to synapse-specific acceleration of the EPSP decay time course. EPSPs with passive dendrites (blue), K⁺ channels in the apical dendrites (black), and K⁺ channels in the basal dendrites (gray). Lower right graph shows normalized superposition. Synapse was located on the distal apical dendrite in all cases. Synaptic peak conductance was 1 to 10 nS. (D) Corresponding plot of EPSP half-duration against synaptic peak conductance. (E and F) Dendritic K⁺ channels enable synapse-specific coincidence detector properties. Plot of paired pulse summation ratio (EPSP_{max}/EPSP₁) for dual activation of the same synapse [(E), continuous lines, distal apical dendrite; and dashed lines, basal dendrite] and activation of different spatially separated synapses (F). Corresponding traces are shown on top. Blue, passive dendrites; black, dendrites enriched with K⁺ channels.

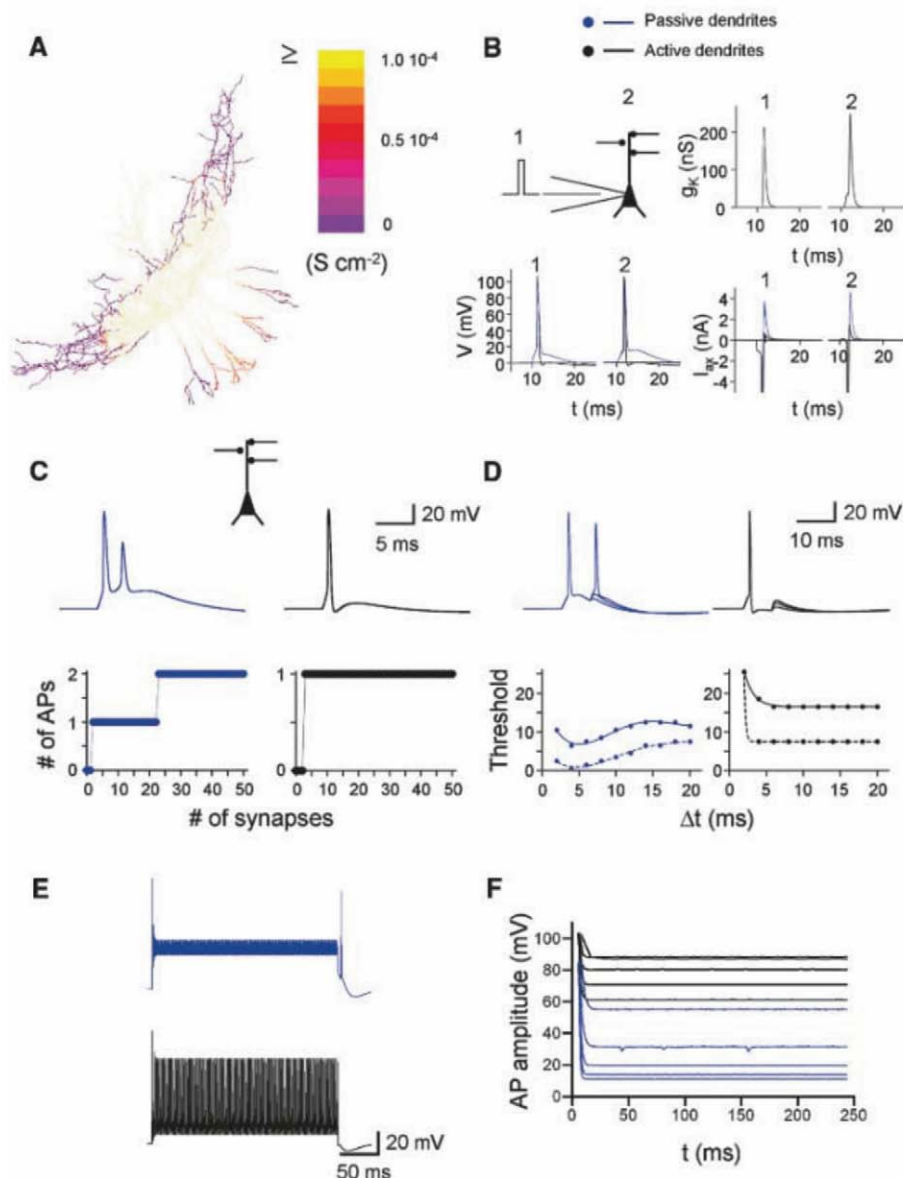


Fig. 6. Dendritic K^+ channels control EPSP-AP coupling and AP phenotype of BCs. **(A)** Simulation of K^+ channel activation in a BC 2 ms after the onset of a somatic current stimulus triggering an AP. Color code (right) shows density of activated K^+ conductance. **(B)** APs (bottom left), total activated dendritic K^+ conductance (top right), and axial current in all primary dendrites (bottom right; negative peak truncated) versus time. APs were initiated either by brief somatic current pulses (2 ms, 1.2 nA, “1”) or by simultaneous activation of 10 synapses randomly placed on apical dendrites (“2”). Note that dendritic K^+ channels abolished the afterdepolarization by reducing the dendrosomatic current flow (positive values of axial current, I_{ax}). **(C)** Dendritic K^+ channels ensure 1:1 EPSP-AP coupling over a wide range of synaptic strength. (Top) APs evoked by simultaneous activation of 50 synapses. (Bottom) Plots of number of evoked APs against number of activated synapses. **(D)** Dendritic K^+ channels normalize the AP threshold for paired synaptic activation. (Top) APs evoked by consecutive activation of two sets of synapses on different dendrites (10 and 1 to 3 active synapses, respectively). (Bottom) Plots of threshold for AP initiation (in units of number of synapses) against time interval between EPSPs. Continuous lines, dual activation of the same synapse; dashed lines, activation of synapses on different dendrites. **(E)** Trains of APs evoked by 250-ms 2-nA depolarizing current pulses for passive and active dendrites. **(F)** Plot of AP amplitude, measured from the peak of AP to the trough of the subsequent afterpotential, versus time for different stimulus intensities (0.5 nA to 2.5 nA in 0.5-nA increments). Blue, passive dendrites; black, active dendrites. In all simulations, voltage given refers to the soma.

tiation threshold during repetitive stimulation. These properties are relevant during in vivo network activity, for example, during sequential ac-

tivation of feedforward and feedback inputs on BCs (41) or sequential activation of presynaptic granule cells with adjacent place fields converg-

ing on the same BC during movement (42, 43). At the output level, dendritic K^+ channels contribute to the fast-spiking AP phenotype (25), enhancing AP repolarization and thereby promoting recovery of Na^+ channels from inactivation. Dendritic channels represent an almost infinite pool, which can be efficiently recruited during physiological and pathophysiological AP activity. After APs, dendritic channels will be activated with longer delays than somatic channels with identical gating, enhancing their delayed rectifier properties. Whether and how dendritic K^+ channels also contribute to the complex (sometimes anti-Hebbian) induction rules for long-term potentiation at glutamatergic principal neuron-interneuron synapses remains to be determined (16, 44).

Because the dendrites of BCs differ significantly from those of somatostatin-expressing interneurons (30, 31), our results demonstrate that the diversity of GABAergic interneurons extends to the dendritic level. Previous results suggested that synaptic facilitation or depression and passive cable properties underlie the “routing” that leads to a switch from perisomatic to dendritic inhibition during repetitive activity (9). Our findings suggest that dendritic properties may participate in this dynamic switch. In fast-spiking, parvalbumin-expressing BCs, limited synaptic dynamics (14, 16) and dendritic K^+ channel activation will facilitate stimulus-locked AP generation in the early phase of repetitive input synapse stimulation. In contrast, in somatostatin-positive interneurons, synaptic facilitation (45–47) and a high Na^+ channel density (30) will promote asynchronous AP generation late in the train. Thus, dendritic properties may contribute to setting the rules for routing of activity in inhibitory microcircuits.

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REPORTS

An Unusually Fast-Evolving Supernova

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Analyses of supernovae (SNe) have revealed two main types of progenitors: exploding white dwarfs and collapsing massive stars. Here we describe SN 2002bj, which stands out as different from any SN reported to date. Its light curve rose and declined very rapidly, yet reached a peak intrinsic brightness greater than -18 magnitude. A spectrum obtained 7 days after discovery shows the presence of helium and intermediate-mass elements, yet no clear hydrogen or iron-peak elements. The spectrum only barely resembles that of a type Ia SN, with added carbon and helium. Its properties suggest that SN 2002bj may be representative of a class of progenitors that previously has been only hypothesized: a helium detonation on a white dwarf, ejecting a small envelope of material. New surveys should find many such objects, despite their scarcity.

Supernovae (SNe) are usually classified on the basis of telltale lines in their spectra (1). Those empirical types are routinely associated with progenitor systems according to the current understanding of their explosion mechanisms. Type Ia SNe are interpreted as the thermonuclear disruption of a white dwarf, and the other types are interpreted as the core collapse of a massive star. SN 2002bj, which we describe here, would formally belong, according to that classification, to the type Ib class because of the lack of H and the presence of He in the optical spectra we have obtained. However, the overall observed properties of this SN are unprecedented, and the taxonomic classification is misleading.

SN 2002bj was discovered independently at magnitude (mag) 14.7 by the Lick Observatory SN Search (LOSS) and by amateur astronomers (2) on 28.2 February 2002 (universal time dates are used throughout this paper) in the galaxy NGC 1821. The distance, corrected for local bulk flows (assuming a Hubble constant of $73 \text{ km s}^{-1} \text{ Mpc}^{-1}$), is $50 \pm 5 \text{ Mpc}$ [see supporting online material (SOM) for a discussion of the host galaxy properties and distance]. A pre-discovery LOSS image with limiting magnitude 18.4 (Galactic extinction corrected) on 21.2 February 2002 (a week before discovery) shows nothing at that position.

As part of our SN followup program, we obtained optical broadband photometry of SN 2002bj in the B, V, R, and I bands for nine epochs over 20 days until it faded below the detection threshold (SOM). Our photometry does not show a rising phase, but the nondetection constrains the rise to be less than 7 days long. The decline was almost as fast, dropping by 4.5 mag (in the B band) in 18 days. SN 2002bj evolves on unprecedented time scales (Fig. 1).

The spectrum we obtained a week after detection is extremely blue, with weak yet remarkable features (3). Using a χ^2 fit, we have digitally compared our (continuum-removed) spectrum with about 4000 spectra of nearly 1400 SNe, allowing for velocity offsets. Not a single spectrum fits well. The closest matches were SNe Ia, mostly due to the absorption feature near 6150 Å (rest frame), usually attributed to Si II (Fig. 2). The best of those (SN 2009dc) is a superluminous, slowly declining, C-rich, possibly super-Chandrasekhar-mass SN Ia (4–6). These few very luminous SNe reported so far evolve slowly and eject substantial amounts of unburned material, suggesting massive white dwarf progenitors. That is, the closest spectroscopic match has one of the most substantially different light curves. Although the spectra are broadly similar, SN 2002bj has prominent He I lines, which are not expected in a SN Ia. In addition, the spectrum of SN 2009dc had to be artificially redshifted by 3000 km s^{-1} in order to match that of SN 2002bj, implying that SN 2002bj had slower ejecta at the time when the spectrum was taken.

Using the code SYNOW (7), we produced synthetic spectra and identified most of the features as coming from helium and intermediate-mass elements such as C, Si, and S, but no H (SOM). Although this empirical fit does not produce meaningful abundances, the lack of Fe or other Fe-peak elements in the fit is peculiar. As exceptional is the considerable S II contribution, when compared to Ca II (a ratio never seen before in other SNe). We also report a tentative identification of V II. Although based on only a single line, the relevant spectral region ($\sim 3950 \text{ Å}$) would have emission from Ca II without it. The spectrum was taken in spectropolarimetry mode, yet there are no polarization line features down to 0.1 to 0.2%. The continuum is consistent with

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polarization by dust in the Milky Way (see SOM for details).

Using our photometry, we determined the bolometric evolution of SN 2002bj (SOM). The total radiated energy in optical bandpasses was on the order of 10^{49} erg, starting at a peak of 10^{43} erg s^{-1} . Assuming blackbody emission, we derived the temporal evolution of the effective temperature, radius, and photospheric velocity. The temperature and velocity declined very rapidly, indicating rapid recession of the photosphere in a low-mass envelope. We estimated the mass of the ejecta using the scaling relation that ties it to the photospheric velocity and rise time $M_{ej,1} = (\frac{v_1}{v_2})^2 \frac{t_1}{t_2} M_{ej,2}$ (8). This scaling assumes

that the opacity is similar to that of a SN Ia. SN 2002bj rose at least three times faster than a normal SN Ia (depending on the assumed explosion date); thus, although its velocity at peak is uncertain (see discussion in SOM), the ejected mass has to be smaller than ~ 0.15 solar mass ($M_\odot$), about 10% that of a SN Ia.

The luminosity and short rise time of SN 2002bj translate to 0.15 to 0.25 $M_\odot$ of ^{56}Ni when using Arnett's law (8, 9), if the light curve is solely powered by radioactive ^{56}Ni and its decay product ^{56}Co . Under this same assumption, the rapid decline we measured requires a sharp drop of the gamma-ray deposition efficiency of an order of magnitude in less than 3 weeks.

Fig. 1. Comparison of the light curve of SN 2002bj to those of SNe of various types (gray dashed lines; R-band magnitudes offset to the same B-band maximum date). SN 2002bj is quite luminous at peak for a core-collapse event, yet faint compared with typical SNe Ia. SN 1994I (21) is often cited as a "fast" SN Ic; SN 2003gs (22) was recently presented as one of the fastest SNe Ia; and SN 2008ha (24) is a faint, peculiar, and fast SN of debated breed. SN 2002bj is significantly faster than any of these. SN 1998S (23), SN 2005cf (24), and SN 2008D (25) are standard representatives of type IIn, Ia, and Ib SNe, respectively; they are shown for reference. The dashed red line shows the slowest rise slope of SN 2002bj allowed by the data.

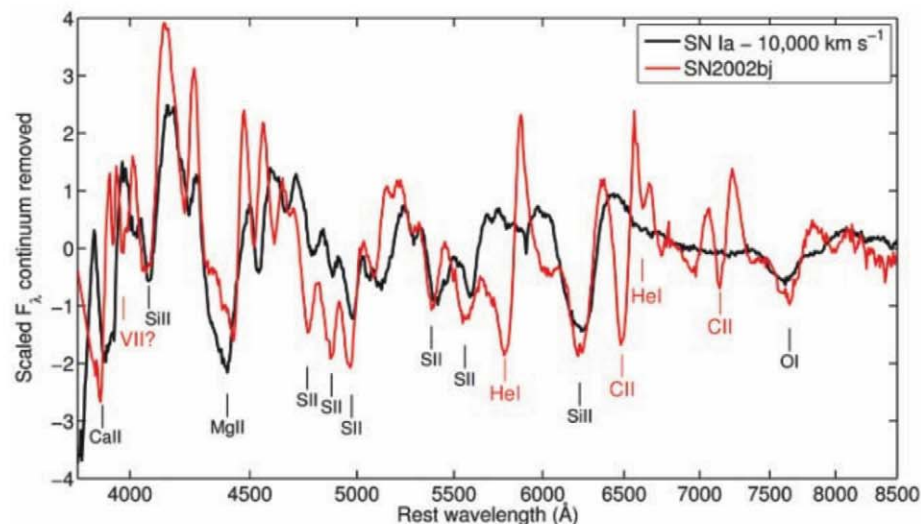
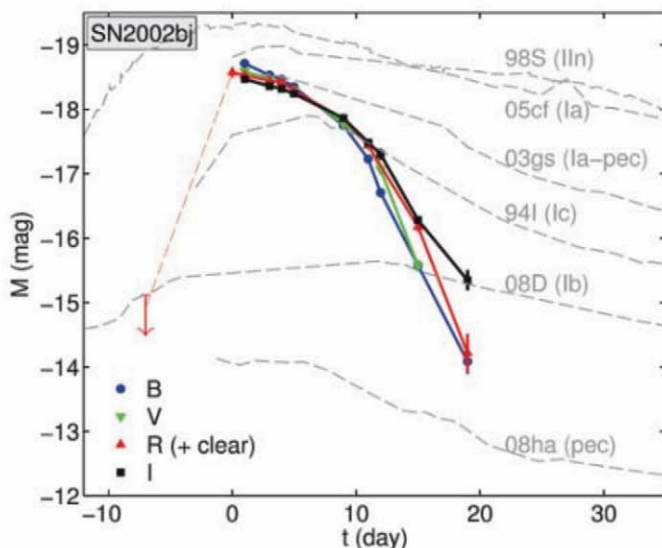


Fig. 2. The unique spectral features of SN 2002bj (shown in red; continuum removed) are difficult to identify a priori. F_λ , flux per unit of wavelength. This spectrum, taken 7 March 2002 (7 days after discovery), is reminiscent of SNe Ia, with the notable exception of the prominent He and C lines, never seen before in such SNe. We show (in black) a typical SN Ia spectrum near maximum light (SN 2001bf), redshifted by $10,000 \text{ km s}^{-1}$ in order to match ejecta velocities. The spectral features identified in black are present in both objects, and the ones in red are seen only in SN 2002bj.

The small ejected mass we derived, the lack of Fe-peak elements in the spectrum, and the relatively large amount of ^{56}Ni required to explain the high luminosity are difficult to reconcile without assuming an additional energy source.

SN 2002bj looks spectroscopically somewhat like a SN Ia but with He, C, and an exceptionally fast light curve. Recently, a mechanism has been proposed (10) by which binary white dwarfs of the AM CVn class may undergo a thermonuclear explosion of the He accreted on the primary star. Such a scenario will produce roughly 10% of the luminosity of a SN Ia, for about 10% of the typical time; hence, these objects were dubbed "Ia" SNe.

These SNe are expected to be faint (between -15 and -18 mag at peak in the V band) and rapidly evolving (1 to 6 days of rise time, with the brighter objects usually rising more slowly). The decline was not explicitly discussed by Bildsten *et al.* (10), but the low ejected mass implies a rapid decline. The short time scales of these events may allow the detection of the short-lived radioactive nuclei ^{52}Fe or ^{48}Cr , in addition to the standard ^{56}Ni that drives SN Ia light curves. ^{48}Cr decays to ^{48}V within a day and then to ^{48}Ti in a week. The decay of these nuclei may (partially) power the optical light curve. The rate of Ia events is predicted to be roughly a few percent of the SN Ia rate per unit of local volume.

The spectral signature was not predicted, but some properties seem to result naturally in that scenario. Because this is a thermonuclear He detonation on a white dwarf, we do not expect any H, but He does seem reasonable, as well as intermediate-mass elements that either survive the convective burning phase and detonation (11) or are produced in the explosion.

Although other recent SNe have been proposed to be related to He detonations on a white dwarf [SN 2005E (12) and perhaps also SN 2008ha (12–14)], these events have more massive ejecta (0.2 to $0.3 M_\odot$) and much slower light curves, and thus do not fit the current predictions of Ia models, though they may be explained with related phenomena involving much more massive He shells.

The light curve of SN 2002bj is as fast as predicted in this model but slightly more luminous than expected (15). The high luminosity yet small ejecta mass may be reconciled if some short-lived ^{48}Cr or ^{52}Fe are synthesized, as their yield per unit of mass is higher than that of ^{56}Ni on short time scales. Our tentative identification of V II in the spectrum supports this hypothesis (^{48}V is the daughter of ^{48}Cr), and the peculiar composition of the spectrum may support it, as well. SNe Ia usually display a prominent secondary peak in their infrared light curve, attributed to line-blanketing by singly ionized Fe-peak elements (16). The lack of a secondary peak in the light curve of SN 2002bj is consistent, within that picture, with our non-detection of Fe-peak elements in the spectrum.

Bildsten *et al.* (10) assumed that the ejecta will have velocities of $\sim 15,000 \text{ km s}^{-1}$, which is

equivalent to all of the binding energy released from fusing He converted into kinetic energy (because the star is assumed to be left bound). We infer for SN 2002bj photospheric velocities that drop rapidly from about 8400 km s^{-1} at detection to 2000 km s^{-1} 3 weeks later. Extrapolating to an explosion date 7 days before detection, the initial velocity could have been between $14,000 \text{ km s}^{-1}$ (linear extrapolation) and $\sim 25,000 \text{ km s}^{-1}$ [exponential extrapolation as often seen in SNe (17, 18)]. A rise time that is faster by a factor of 2 would imply velocities twice as high. The only direct measurement we have is from the spectrum 7 days after detection: about 4000 km s^{-1} . This is consistent with the derived photospheric velocity at that time if the rise time was about 7 days.

Out to a distance of 60 Mpc, the LOSS survey is complete (99%) for SNe Ia, and 31 have been found. Because SN 2002bj is quite luminous, the incompleteness correction for it is almost as small (94%), resulting in a relative rate of 3.4% of the SN Ia rate for SN 2002bj-like SNe (19). This is in good agreement with the predictions for SNe Ia.

The SN Ia model, still in its infancy, lacks more stringent predictions such as detailed light curves and spectral composition and evolution. Nevertheless, all the diagnostics we could apply seem consistent with this interpretation. The evidence here is tentative, but the existence of V, if seen in future discoveries of objects of this class, points to a different nucleosynthetic chain and

therefore may serve as a smoking gun for a truly different SN explosion channel. Regardless of the interpretation, current and future surveys should focus on short cadences—repeat visits on daily rather than weekly time scales—in order to find many more SNe resembling SN 2002bj.

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SOM Text
Figs. S1 to S4
Tables S1 to S3
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Polarization-Induced Hole Doping in Wide-Band-Gap Uniaxial Semiconductor Heterostructures

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Impurity-based p-type doping in wide-band-gap semiconductors is inefficient at room temperature for applications such as lasers because the positive-charge carriers (holes) have a large thermal activation energy. We demonstrate high-efficiency p-type doping by ionizing acceptor dopants using the built-in electronic polarization in bulk uniaxial semiconductor crystals. Because the mobile hole gases are field-ionized, they are robust to thermal freezeout effects and lead to major improvements in p-type electrical conductivity. The new doping technique results in improved optical emission efficiency in prototype ultraviolet light-emitting-diode structures. Polarization-induced doping provides an attractive solution to both p- and n-type doping problems in wide-band-gap semiconductors and offers an unconventional path for the development of solid-state deep-ultraviolet optoelectronic devices and wide-band-gap bipolar electronic devices of the future.

The direct-gap III-V nitride semiconductor family and its alloys span the widest spectral range of band gaps (E_g) among all semiconductors, ranging from the infrared (InN, $E_g = 0.7 \text{ eV}$) through the visible and the ultraviolet (UV) (GaN, $E_g = 3.4 \text{ eV}$) to the deep UV range (AlN, $E_g = 6.2 \text{ eV}$). This property is the basis for its applications in short-wavelength

lasers (1, 2) and in light-emitting diodes (LEDs) for solid-state lighting applications (3, 4). In addition, the wide band gaps, availability of heterojunctions, high electron-saturation velocities, and high breakdown fields enable high-speed and high-power electronic devices. Compact short-wavelength, solid-state light sources will enable a wide range of applications such as high-density

optical data storage, water treatment, sterilization of medical equipment, UV-enabled security marks on credit cards and currency bills, and biological and cellular imaging.

Currently, the III-V nitride semiconductors offer the most viable approach toward the realization of high-efficiency, deep-UV optical emitters based on semiconductors (2). A problem that has persisted since the early 1990s and is becoming increasingly troublesome is the high resistivity of p-type GaN and AlGaN layers. The activation energy E_A of the most commonly used acceptor dopant (Mg) in GaN is $\sim 200 \text{ meV}$ (5–7), several times the thermal energy $k_B T$ at room temperature (where k_B is the Boltzmann constant, and T is temperature). The activation energy of acceptors increases with the band gap, reaching $E_A \sim 630 \text{ meV}$ in AlN (1). For comparison, the donor (Si) activation energies are $E_D \sim 15 \text{ meV}$ for GaN and $E_D \sim 282 \text{ meV}$ for AlN (1). Thus, the thermal activation of holes is highly inefficient at room temperature for GaN and becomes increasingly problematic for higher-band-gap AlGaN and AlN layers. As a result, injection of holes is a severe impediment for light-emitting devices in

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the UV and deep-UV spectral windows. High p-type resistance leads to excessive Joule heating of p-doped AlGa_N layers for Al composition $x_{\text{Al}} \geq 20\%$. Instead, p-GaN layers must be used and absorption losses incurred in the narrower-band-gap region. Furthermore, hole reflection and trapping at heterojunction valence-band offsets block hole injection into optically active AlGa_N regions (2) and reduce the efficiency of such devices. An alternative strategy for efficient p-type doping and hole injection in wide-band-gap semiconductors is therefore highly desirable at this time.

The large ionic component of the Ga(Al)-N bonds, combined with the deviation of their equilibrium lattice structure from ideal wurtzite crystals, give rise to giant spontaneous polarization fields in III-V nitride semiconductors (8, 9). In addition, the strain-induced piezoelectric component of the fixed charge in the nitrides is the highest among all III-V semiconductors. At abrupt Al(Ga)/N/GaN heterojunctions, the sharp discontinuity in the polarization field leads to the formation of a bound sheet charge σ_{π} at the heterointerface, captured by the Gauss law boundary condition $\sigma_{\pi} = (\mathbf{P}_1 - \mathbf{P}_2) \cdot \hat{\mathbf{n}}$, where $\hat{\mathbf{n}}$ is the unit vector normal to the heterointerface, and $(\mathbf{P}_1, \mathbf{P}_2)$ are the polarization fields across the heterojunction. When wurtzite nitride crystals are grown along the [0001] orientation (metal or

Ga-face), a positive bound polarization charge creates a high electric field and energy-band bending, such that a mobile two-dimensional electron gas (2DEG) forms at AlGa_N/GaN heterojunctions without the need for intentionally introduced impurity dopants. The bound sheet-charge density can be as high as $\sigma_{\pi} \sim 6 \times 10^{13} \text{ cm}^{-2}$ at pseudomorphic AlN/GaN heterojunctions, facilitating mobile 2DEGs with a very high charge carrier density. For example, in AlN/GaN semiconductor heterostructures, the mobile 2DEG concentrations are $4 \times 10^{13} \text{ cm}^{-2}$ (10). Such polarization-induced 2DEGs form the basis of nitride high-electron mobility transistors that have surpassed transistors made from any other semiconductor family in RF power performance (11).

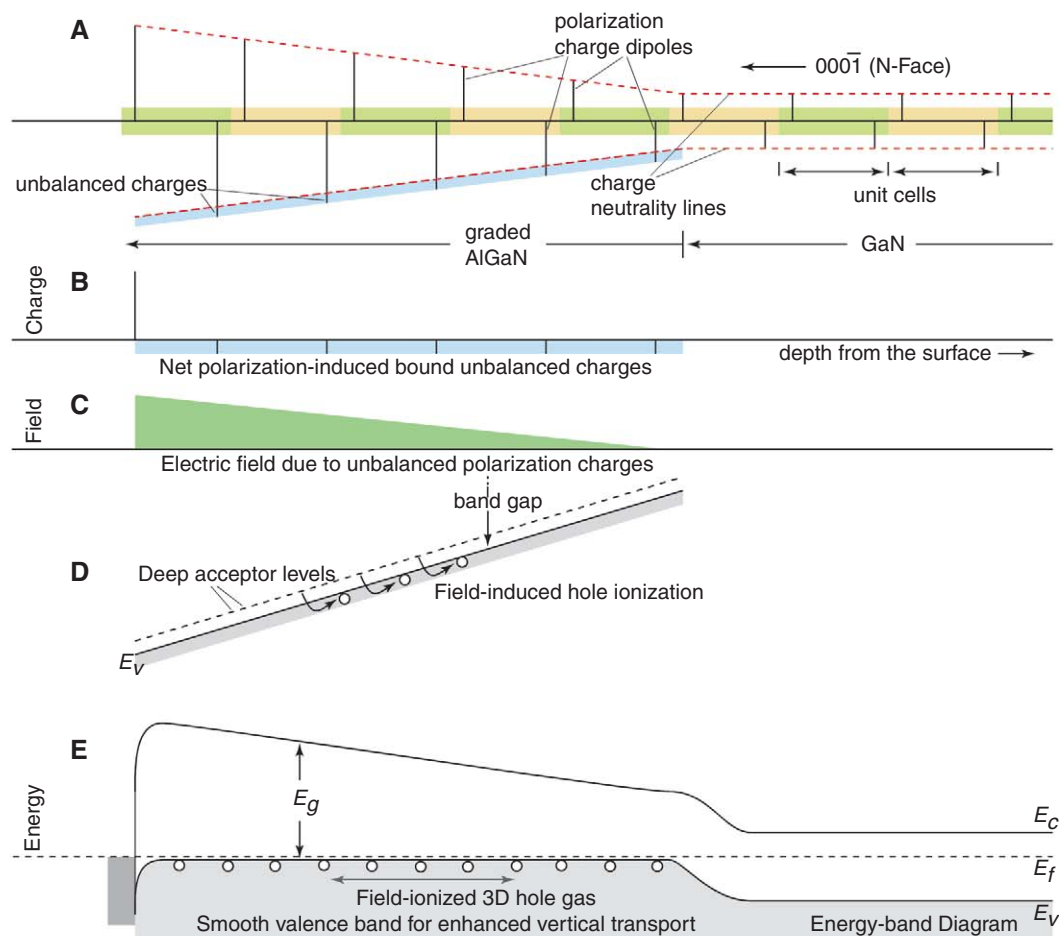
The polarization fields have also been exploited to create parallel sheets of 2D hole gases in Mg-doped AlGa_N/GaN multiple-quantum-well structures (12, 13). Although such parallel 2D hole sheets have high conductivity in the plane of the heterojunctions, they suffer from low conductivity perpendicular to the interfaces because of potential barriers in the valence band that require transport to occur through tunneling or thermionic emission processes. Even in short-period superlattice structures, the large effective mass of holes in minibands results in low mobility and high resistance (1). An alternate strat-

egy for hole doping without potential barriers will facilitate higher conductivities.

If instead of sharp heterojunctions we grew a compositionally graded crystal, the bound polarization-induced sheet charge spreads to a bound 3D form in accordance with $\rho_{\pi}(z) = -\nabla \cdot \mathbf{P}(z)$, where $\rho_{\pi}(z)$ is the volume charge density in the polar (z) direction, and $\nabla \cdot$ is the divergence operator. For [0001]-oriented Ga-face crystals graded from GaN to AlGa_N, the polarization bound charge is positive and induces the formation of a mobile 3D electron gas. These 3D electron slabs are quite distinct from those formed by donor impurity doping: Because the carriers are created by effective electrostatic “field” ionization, they require no impurity incorporation, and thus exhibit virtually no freezeout at cryogenic temperatures as opposed to thermally ionized carriers in shallow, donor-doped layers (14). The resulting 3D electron gases have higher n-type conductivity than impurity-doped layers of comparable carrier concentration, because ionized impurity scattering is absent. The absence of freezeout and high mobilities made it possible to observe Shubnikov–de Haas oscillations (15). Polarization-induced field-effect transistors have also been demonstrated recently with this technique (16).

By the same measure, flipping the polarity of the crystal (growing along the N-face, which is

Fig. 1. Schematic illustration of polarization-induced p-type doping in graded polar heterostructures. (A) Sheets of charge dipoles in every unit cell of the crystal. The net unbalanced polarization charge is shown in (B), which leads to the electric field in (C), and the energy-band bending in the valence band in (D) if holes are not ionized. Field ionization of holes results in a steady-state energy-band diagram shown in (E), which highlights the smooth valence-band edge without any potential barriers for hole flow. E_f is the Fermi level; E_c and E_v are the conduction and valence-band edges, respectively; and E_g is the band gap.



the $[000\bar{1}]$ direction) and compositional grading from GaN to AlGa N should result in mobile 3D hole slabs. The ability to do so without the introduction of Mg-acceptor dopants hinges on the propensity of the surface to act as a remote acceptor state. The surface of III-V nitride semiconductors freely provides mobile electrons, but not holes, and this difference has been attributed to the presence of deep-level traps that localize holes (17). Lowering of defect and trap densities may enable dopant-free p-type carriers, but intentionally introducing Mg-acceptor dopant atoms in the N-face graded AlGa N layer serves as the necessary source of holes. This work demonstrates the ability to use the polarization charges

in N-face $[000\bar{1}]$ layers to generate polarization-induced, p-type graded AlGa N slabs that are highly conductive.

The mechanism of polarization-induced hole formation is illustrated schematically in Fig. 1. The total polarization (spontaneous plus piezoelectric) can be pictured as charge dipoles in every unit cell of the crystal (Fig. 1A). Because Al $_x$ Ga $_{1-x}$ N (where x is the Al mole fraction) has higher polarization than GaN, the sheet-charge dipoles in unit cells of the AlGa N layer are of a higher magnitude than in GaN, so the dipole strength increases linearly with the composition. When the composition of the layer is graded with increasing Al mole fraction, the net unbalanced

bound polarization charge is negative (Fig. 1B), given by $\rho_\pi(z) = -\nabla \cdot \mathbf{P}(z) \sim 5 \times 10^{13} \times (x_2 - x_1)/d$ cm $^{-3}$, where x_1 and x_2 are the Al compositions at the ends of the graded layer of thickness d (in centimeters). This bound charge creates a built-in electric field (Fig. 1C) and energy-band bending that would be greater than the band gap of the semiconductor layer if left uncompensated (Fig. 1D). To neutralize the bound, negative polarization charge, holes are consequently field-ionized from the deep Mg-acceptor atoms and form a high-density mobile 3D hole gas. The concentration of the 3D hole gas should then exhibit a weak temperature dependence and resist freezeout at low temperatures. In addition, the

Fig. 2. Structural and transport properties of p-type samples (A) Concentration of Al and Ga atoms in a compositionally graded AlGa N sample (sample d), with the measured concentration of Mg dopant atoms. The thickness of the graded layer is $d \sim 85$ nm, capped with a thin, heavily doped p++ layer for ohmic contacts. SIMS, secondary ion mass spectrometry. (B) Measured temperature-dependent resistivity for samples a to c, highlighting the polarization boost in p-type conductivity.

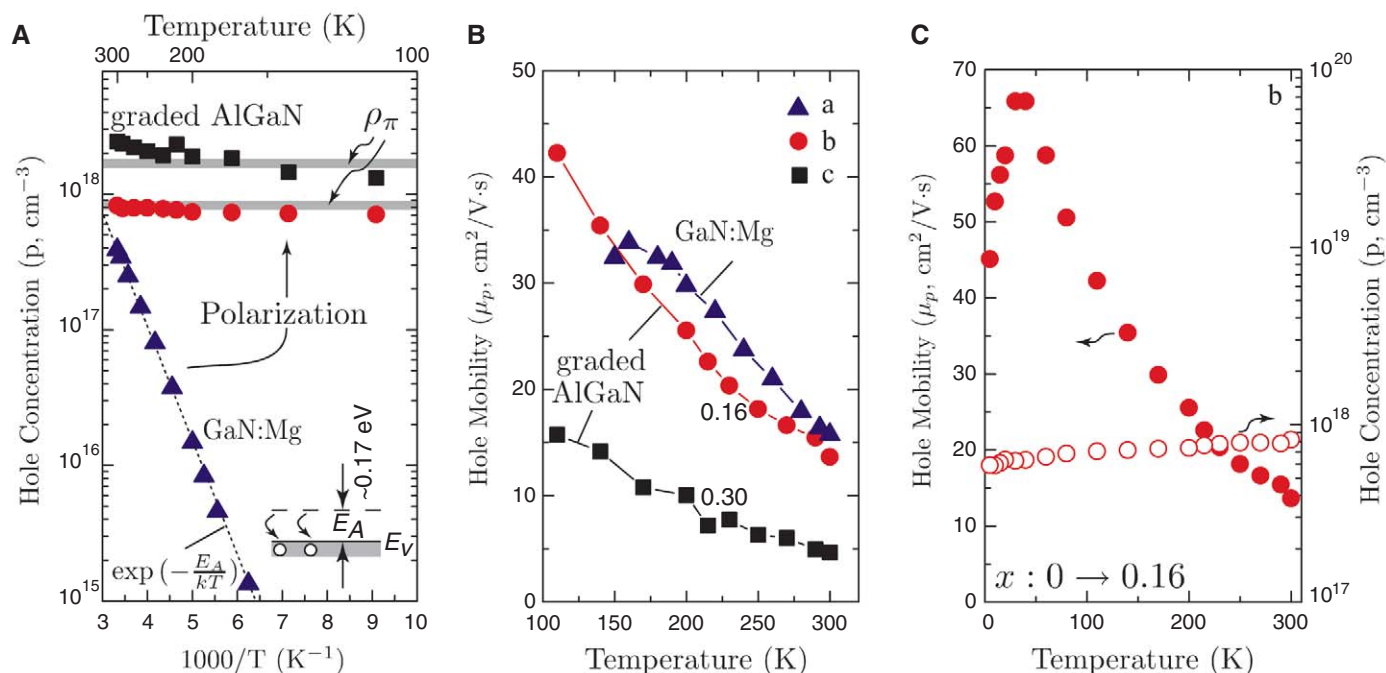
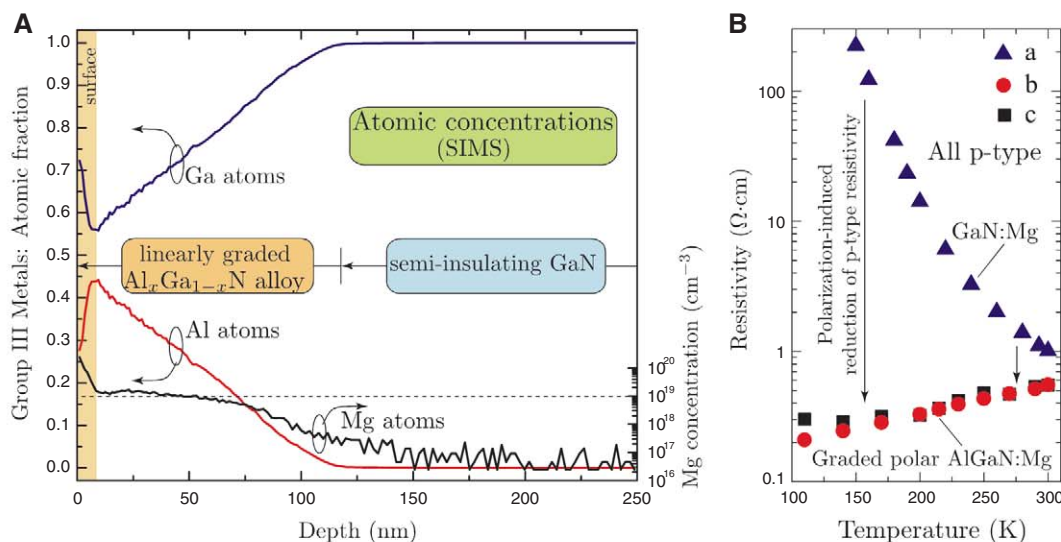


Fig. 3. Hall-effect temperature-dependent (A) hole concentration, (B) hole mobilities, and (C) hole concentration and mobility measured down to $T = 4$ K. The polarization-doped graded AlGa N p-type layers show higher hole

concentrations and conductivities. Holes in polarization-doped layers are resistant to freezeout at low temperatures, and their mobility and concentration can be measured down to cryogenic temperatures.

smooth spatial variation of the valence-band edge (Fig. 1E) should further facilitate high-conductivity p-type transport in both lateral and vertical directions.

To test this concept of polarization-induced p-type doping, Mg-doped graded AlGaIn layers were grown on top of semi-insulating N-face [0001] GaN substrates by plasma-assisted molecular beam epitaxy. A Mg-doped GaN sample ($N_a \sim 10^{19}/\text{cm}^3$, sample a; here, N_a is the acceptor concentration) was used as a control sample. Graded AlGaIn samples doped with the same Mg concentration but linearly graded from $x = 0$ to 0.16 (sample b) and $x = 0$ to 0.3 (sample c) over $d \sim 85$ nm were grown. The sample structures and compositions were characterized by x-ray diffraction, in situ reflection, high-energy electron diffraction patterns and atomic force microscopy [see supporting online material (SOM) for a description of crystal growth and characterization (18)]. Secondary ion mass spectrometry measurements were performed on a separate graded AlGaIn sample ($x = 0$ to 0.4, sample d) as well as the control Mg-doped GaN sample to verify the incorporation of Mg atoms into the crystal and the linear grading of Al composition in the polarization-doped AlGaIn layers (Fig. 2A). Samples a to c were subsequently processed for Hall-effect measurements, as described in the SOM (18).

The measured resistivities of the bulk p-GaN and polarization-doped AlGaIn layers from $T = 300$ to 100 K are shown in Fig. 2B. The room-temperature resistivity of both polarization-doped samples b and c ($\rho_b, \rho_c \sim 0.6$ ohm-cm) is lowered by more than a factor of 2 compared with that of the control sample a ($\rho_a \sim 1.22$ ohm-cm). The

resistivity of the control sample a increased monotonically by more than two orders of magnitude as the temperature was lowered from 300 to 100 K (Fig. 2B); this increase is expected from the freezeout of thermally activated holes. In comparison, the resistivities of the polarization-doped samples b and c actually decreased with temperature, which is indicative of metallic behavior. This decrease in resistivity can occur if (i) polarization-induced holes do not freeze out at low temperatures and (ii) the mobilities of polarization-induced holes increase when temperature is lowered from 300 to 100 K.

Temperature-dependent Hall-effect measurements performed at a magnetic field of 0.5 T confirmed the two hypotheses. The measured hole concentrations and mobilities are shown in Fig. 3, A and B. Compared with the exponential freeze-out (activation energy $E_A \sim 170$ meV) of mobile holes for the Mg-doped GaN control sample (a), the hole densities in the polarization-induced graded AlGaIn samples (b and c) remain essentially independent of temperature, and are near the theoretical prediction [$p_\pi \sim 5 \times 10^{13} \times (x_2 - x_1)/d$ cm $^{-3}$], as indicated by the thick gray lines in Fig. 3A. Polarization charges are atomic in origin and do not require thermal energy to be activated, so they enhance the hole concentration independent of temperature. In addition, because polarization charges are spatially distributed, the band-edge potential variations are smooth, and no abrupt potential barriers exist for the flow of holes along any direction. These properties are a major advantage and novelty of this method of p-type doping. Polarization enhancement of hole densities are $2\times$ and $6\times$ for samples b and c at room temperature and many orders of magnitude at lower temperatures.

The measured hole mobilities as a function of temperature in samples a to c are shown in Fig. 3B. Sample c has lower hole mobility because of increased alloy scattering. Although it was not possible to perform Hall-effect measurements for control sample a below $T \sim 150$ K because of carrier freezeout, we measured the polarization-enhanced hole concentration and mobility down to $T = 4$ K for sample b. As shown in Fig. 3C, the hole concentration showed a very small decrease with temperature, whereas the hole mobility increased to $\mu_p \sim 65$ cm $^2/\text{V}\cdot\text{s}$ at 30 K before decreasing, indicating competition between phonon and impurity scattering.

To test the effectiveness of such polarization-enhanced p-type layers as hole injectors in optical devices, a Mg-doped graded AlGaIn layer ($x = 0$ to 30%, identical to sample c) was grown on a n-type doped N-face [0001]-oriented GaN substrate. A control p-n junction with a Mg-doped GaN p-type layer was also grown, and these structures were fabricated into light-emitting diode structures [see SOM for the fabrication procedure (18)]. These junctions serve as prototype LEDs, requiring electrical injection of holes and electrons into the depletion region where they recombine radiatively to emit photons. Under forward bias at room temperature, both devices exhibit electroluminescence in the UV spectral range (Fig. 4A). We observed characteristic sub-band-gap emission attributed to deep acceptor levels in nitrides (19). Furthermore, we note that the graded AlGaIn p-layer structure showed much brighter optical emission (Fig. 4B), which we attribute to two factors: (i) improved p-type conductivity in the vertical direction due to polarization-induced hole doping and (ii) the existence of a built-in quasi-

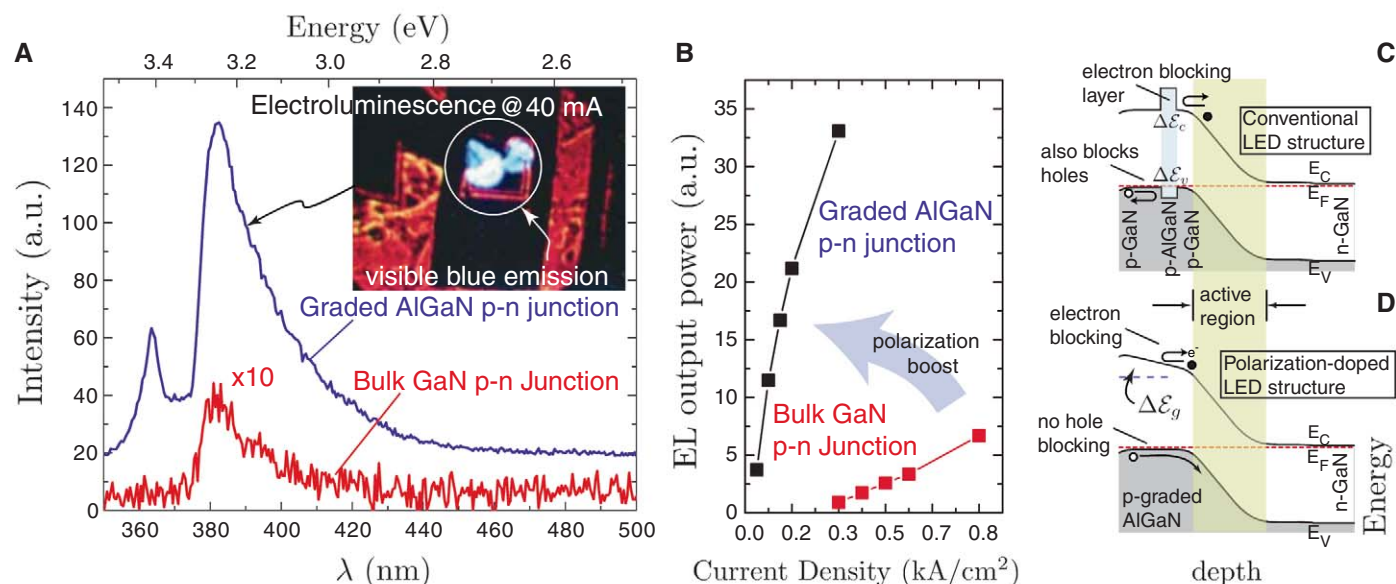


Fig. 4. (A) Room temperature electroluminescence of the graded p-type AlGaIn junction and the control GaN p-n junction at 40-mA drive current. Both samples have an area of $80 \mu\text{m}$ by $150 \mu\text{m}$. (Inset) Optical microscope micrograph displaying the blue part of the emission of the graded AlGaIn junction. a.u., arbitrary units. (B) Relative output intensity with increasing drive current for the graded AlGaIn p-n junction and the control bulk-doped p-n junction. The

polarization-doped diode shows much brighter emission than the bulk-doped p-n junction. Schematic energy-band diagrams of a conventional (C) LED device and (D) a polarization-doped device. The graded AlGaIn p-n junction uses the entire band offset ΔE_g in the conduction band as an electron-blocking layer, resulting in enhanced electroluminescence. In comparison, a traditional electron-blocking layer (C) also blocks holes through a valence-band offset ΔE_v .

electric field imposed on minority electrons injected into the p-type layer of the graded AlGaIn. The compositional grading in the p-type AlGaIn layer causes the increase in the band gap ΔE_g to appear entirely in the conduction band, which acts as a natural electron-blocking layer. This feature is shown in the energy-band diagram in Fig. 4D. Electron-blocking layers have been shown to improve the efficiency of emission by preventing the spillover of higher mobility electrons from the optically active regions of nitride LEDs (20). Conventional electron-blocking layers implemented in nitride LEDs and lasers consist of a thin AlGaIn layer placed on the p-doped side [schematic band diagram shown in Fig. 4C]. In addition to blocking electron overflow through a conduction band barrier ΔE_C , such layers also prevent efficient hole injection because of the unavoidable valence-band offset ΔE_V (21). Polarization-doped graded layers provide a solution to this design bottleneck. In addition to improving the p-type conductivity, the polarization-induced graded p-type AlGaIn layer facilitates electron blocking without adding barriers to hole injection and offers an added degree of freedom in graded-refractive-index design, all of which are useful for UV laser applications. The polarization-doped layer is also of a larger band gap than the active region of the p-n junction and serves as a natural optically transparent layer with minimal absorption losses.

This method of polarization doping should prove particularly useful for deep-UV optoelectronic applications where both p- and n-type doping of high Al composition AlGaIn is a major challenge. The technique presented here could be applied to produce highly conductive p-type regions in wide-band-gap nitrides composed of high-Al composition AlGaIn and in the more general AlInGaIn material system with proper choice of the crystal direction of growth and management of strain within allowable limits. The doping scheme can be used to obtain desired hole or electron concentrations in spite of poor ionization efficiencies of deep-level dopants in any semiconductor crystals that possess sufficiently strong spontaneous and piezoelectric polarization (for example, in the ZnO material family).

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Supporting Online Material

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Materials and Methods

Figs. S1 to S3

References

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Translocation of Single-Stranded DNA Through Single-Walled Carbon Nanotubes

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We report the fabrication of devices in which one single-walled carbon nanotube spans a barrier between two fluid reservoirs, enabling direct electrical measurement of ion transport through the tube. A fraction of the tubes pass anomalously high ionic currents. Electrophoretic transport of small single-stranded DNA oligomers through these tubes is marked by large transient increases in ion current and was confirmed by polymerase chain reaction analysis. Each current pulse contains about 10^7 charges, an enormous amplification of the translocated charge. Carbon nanotubes simplify the construction of nanopores, permit new types of electrical measurements, and may open avenues for control of DNA translocation.

We report the use of single-walled carbon nanotubes (SWCNTs) as nanopores for analyzing molecular transport properties. Nanopores are orifices of molecular diameter that connect two fluid reservoirs. At this length scale, the passage of even a single molecule generates a detectable change in the flow of ionic current through the pore (1, 2). They can be used as single-molecule Coulter counters and form the basis of proposed new approaches to DNA sequencing (3). The first nanopore devices were based on pore proteins (4–7), but more

recently pores have been fabricated by drilling (and sometimes partially refilling) solid-state materials (8–12). Nanochannels have been formed by etching silicon nanowires (13), and channels with one nanoscale dimension have been etched into glass (14) or quartz (15).

Carbon nanotubes are obvious candidates for the fabrication of nanopore structures. Pressure-driven gas, water, and ion transport has been recorded through membranes composed of many multiwalled carbon nanotubes (16) or double-walled carbon nanotubes (17). These experi-

ments showed that the water flow rate is greatly enhanced inside the tube, an effect confirmed by molecular dynamics simulations (18). DNA has been passed through a 100-nm diameter carbon nanotube (19) and 50-nm-wide hydrophilic channels (13). It seems counterintuitive that hydrophilic DNA would enter the hydrophobic interior of a SWCNT, but simulations show that both RNA (20) and DNA (21) will translocate through 1.5- to 2-nm diameter tubes. The simulations were carried out using very large electric fields (tenths of a volt per nm) to generate observable motion on the simulation time scale. This result leaves open the possibility that some measurable translocation might occur at the much smaller fields that could be implemented in the laboratory. Here, we report direct measurement of this translocation.

We have made a device in which one SWCNT spans a barrier between two fluid reservoirs [see Fig. 1 and Supporting Online Material (22)]. Relative to CNT membranes (16, 17), this arrangement makes it possible to detect signals from the translocation of a single molecule and to correlate

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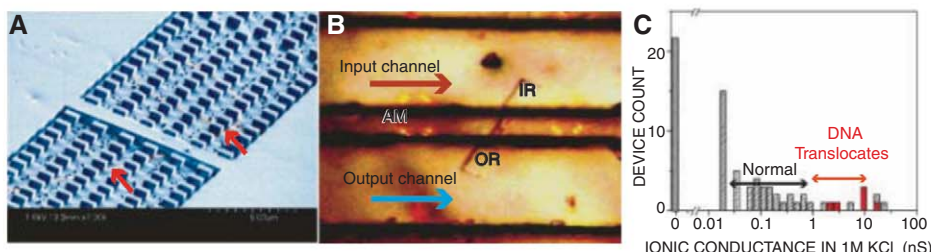


Fig. 1. (A) A single nanopore device was fabricated by growing SWCNTs at low densities on an oxidized Si wafer. We used cobalt catalyst particles with ethanol vapor as the carbon source in conditions most likely to produce high-quality SWCNTs with an outside diameter of 1 to 2 nm (30). A 700-nm layer of PMMA resist is spun on and reservoirs opened over selected tubes with electron-beam lithography. The exposed regions of SWCNTs were removed by O_2 plasma etch. The SEM image of the device shows a 2- μ m barrier before removal of the exposed SWCNT (arrows). Pillars in the reservoir support the PDMS cover. (B) Optical micrograph taken through a PDMS cover. The reservoirs (IR, input; OR, output) span the barrier between PDCS channels at an angle of about 60°. AM marks the location of one set of alignment markers. (C) Current flows through the single SWCNTs and not through a leakage path. With the SWCNT bridging the gap and opened, most tubes pass currents in the expected range (Normal), but 20% pass unexpectedly large currents. Some of these (marked in red) also passed DNA oligomers. These data are limited to the subset of devices exposed to short plasma etches for which control experiments show no leakage (22).

Table 1. Relation of ionic conductance with electrical properties (V_T is the threshold voltage for semiconducting tubes). These measurements do not discriminate between metallic SWCNTs and bundles containing a metallic tube, but most of the tubes are single-walled (30). Raman scattering was used to determine diameters marked * and confirm electronic properties marked †. The tube marked ‡ translocated DNA.

FET device ID	Ionic current	Diameter (nm)	Electrical property
HL_4_1_41 AP3	10.7 ± 0.05 nA/0.4 V	2.0	Metallic
HL_4_1_10 AB6	3.4 ± 0.04 nA/0.5 V	1.7 (1.5*)	Metallic†
HL_4_1_39 P6	2.5 ± 0.07 nA/0.4 V‡	4.2	Metallic
HL_4_1_41 AZ3	1.91 ± 0.05 nA/0.4 V	—	Metallic
HL_4_1_41 N2	0.98 ± 0.04 nA/0.4 V ($V_T \sim 10$ V)	0.9	Semiconducting
HL_4_1_37 AB20	0.46 ± 0.03 nA/0.5 V ($V_T \sim 10$ V)	1.3*	Semiconducting†
HL_4_1_41 AS3	0.07 ± 0.02 nA/0.4 V ($V_T \sim 25$ V)	1.8	Semiconducting
HL_4_1_37 Z22	0.10 ± 0.03 nA/0.5 V ($V_T \sim 10$ V)	1.1*	Semiconducting†
HL_4_1_41 M8	<10 pA/0.4 V($V_T \sim 25$ V)	3.4	Semiconducting

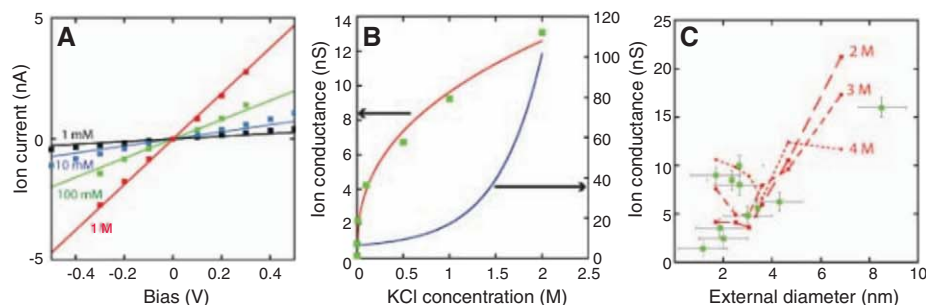


Fig. 2. Ion transport in the subset of SWCNTs with high ionic conductance. (A) Current versus voltage applied to Ag/AgCl reference electrodes for a 2- μ m long, 1.7-nm diameter SWCNT for various concentrations of KCl electrolyte as marked. The solid lines are simulated as described in the text. (B) Ionic conductance as a function of salt concentration. The red line is a fit to the c^m dependence suggested by molecular dynamics simulations. We found $0.33 < m < 0.4$ in three different tubes. The blue line shows the salt dependence of conductance measured in a planar nanopore (27). (C) In this subset of tubes, current at 1 M KCl is better related to diameter (green squares). The red dashed lines show simulations for excess charge densities of 2, 3, and 4 M.

transport with the properties of individual SWCNTs. We grew well-separated SWCNTs on the surface of oxidized silicon wafers and formed fluid reservoirs along the path of chosen tubes with electron-beam lithography. A scanning electron microscopy (SEM) image of a device at this stage is shown in Fig. 1A, where the SWCNT is just visible on each side of the barrier. An oxygen plasma was used to remove the exposed parts of the SWCNT, leaving the SWCNT under the barrier intact (fig. S4I) (22, 23). The fluidic pathway was completed by placing a poly(dimethylsiloxane) (PDMS) cover on top of the chip (Fig. 1B).

Each chip also contained control devices lacking the bridging SWCNT (22) to check the integrity of the barrier, including devices with unopened SWCNTs. We used a mild plasma treatment such that 100% of the devices lacking CNTs did not leak (fig. S5), although this approach resulted in a large fraction of tubes that were not opened (20%), as determined by SEM imaging (fig. S4F). The fluid reservoirs were filled with 1 M KCl, and Ag/AgCl electrodes (BASI MF-2078) were used to measure the conductance across the reservoirs connected by the SWCNT. The devices passed current if, and only if, they were spanned by a SWCNT that was opened (Fig. 1C), so the interface between the tube and the poly(methyl methacrylate) (PMMA) does not appear to provide a leakage path. This conclusion was verified by chemically tethering poly(ethylene glycol) molecules to one or both ends of the CNTs. The current was reduced in one direction of bias when the tube was modified at one end, and in both directions of bias when the tube was modified at both ends (fig. S6).

The ionic conductance of a tube of electrolyte should be given by $G = 6.02 \times 10^{26} (\mu_K + \mu_{Cl}) c_{KCl} e \pi D^2 / 4L$, where $\mu_K = 7.62 \times 10^{-8}$ m²/V s, $\mu_{Cl} = 7.91 \times 10^{-8}$ m²/V s, c_{KCl} is the KCl concentration in mole/l, e the electronic charge, D the tube diameter, and L the tube length. Table 1 shows that there is no correlation between the tube diameter and ionic conductance. The ionic conductance spans nearly four orders of magnitude (Fig. 1C), with only the lowest conductances (the range marked “normal” in Fig. 1C) being consistent with the classical formula for $c_{KCl} = 1$ M, $1 \text{ nm} < D < 5 \text{ nm}$ (fig. S7) and $L = 2 \mu\text{m}$. We also measured the electronic properties of some of the tubes (Table 1) using both their response as field-effect transistors and Raman scattering (figs. S8 to S10). The SWCNTs with the highest ionic conductance are all metallic.

We considered whether the excess current could be accounted for by electrochemical currents stemming from reduction and oxidation reactions at the end of metallic tubes. A conducting tube suspended in a potential gradient in an electrolyte acts as a bipolar electrode (24), but enormous fields are required to drive electrochemical processes at the ends of a bipolar carbon nanotube electrode (25). Measurements with

an electrode contacting the SWCNT directly revealed that electrochemical currents were negligible for the potentials used here (fig. S11). To look for clues to a mechanism for the large ionic currents, we used molecular dynamics simulations coupled with solutions of the Poisson-Nernst-Planck equation for transport in the SWCNT and the outside reservoirs (22). The flow rate of water is greatly enhanced inside SWCNTs (17), but the molecular dynamics simulations showed that the electrophoretic mobility of ions is similar to that in the bulk electrolyte. However, the selective filtering of anions or cat-

ions owing to charged end groups (26) can result in a net excess concentration, n , of one charge inside the tube. This charge will, in turn, drive an electroosmotic current. Molecular dynamics simulations further showed that both water and ions flow with an electroosmotic velocity, v , given by $v \propto n^{0.74}$ for a (10,10) SWCNT. Both anions and cations are driven in the same direction by an extremely large electroosmotic flow, but only the charge imbalance inside the tube results in a net ionic current proportional to nv , that is, $\propto n^{1.74}$. The mechanism of charge accumulation is complex and involves both charged

end groups and the electronic properties of the SWCNT, and we have not yet developed a quantitative model for it (see fig. S12 for further evidence of the role of charged end groups). However, current-voltage curves obtained at different salt concentrations in the reservoirs, c , can be fitted if $n = 3.31 c^{0.22}$ M (Fig. 2A). This result is equivalent to an ionic conductance that varies as $c^{0.39}$, shown by the red curve passing through the measured data in Fig. 2B. This dependence on concentration is quite different from the linear dependence expected for a tube of electrolyte or the saturation at low salt observed for a planar nanopore carrying a surface charge (27).

In contrast to the full set of devices, the subset with anomalously high conductance does show some relation between conductance and tube diameter (Fig. 2C, green squares). The red dashed lines show simulated values of ionic conductance as a function of diameter for $n = 2, 3$, and 4 M. The measured data can be accounted for by assuming that variability in the charge of end groups leads to some variability in n .

Although it might be instructive to study the translocation of simpler polyelectrolytes as a prelude to the study of DNA, methods such as dye-labeling are much less sensitive than polymerase chain reaction (PCR) for detecting and counting small numbers of molecules.

To test for DNA translocation of SWCNTs, we used 60-nt and 120-nt DNA oligomers with sequences that were predicted to be relatively free of secondary structure, with forward and reverse primers chosen to have high melting temperatures to minimize primer dimers and false priming (22). Devices were characterized by measuring current flow with 1 or 2 M KCl alone, and then a DNA solution [1 or 2 M KCl, 1 mM phosphate-buffered saline (PBS), pH 7] was flowed into the input reservoir side. A control sample was collected from the output reservoir to check for DNA contamination, and a positive bias was then applied to the output side of the device. In the subset of high current tubes, we first observed a slow increase in the background current (Fig. 3, A and B; data are for 0.1 nM DNA). After a time, which varied from a few to tens of minutes, depending on the DNA concentration in the input reservoir, large transient increases in current were observed. These “spikes” were accompanied by large fluctuations in the background current (Fig. 3C). The spikes disappeared when the polarity of the bias across the tube was reversed and reappeared when the original bias (positive on the output side) was restored. Quantitative PCR (QPCR) (22) showed that DNA was translocated in devices manifesting these large spikes. Translocation occurred only in tubes with conductances (before DNA addition) of >2 nS (Fig. 1C). Some devices that showed instabilities in the background but no large current spikes (Fig. 3F) gave negative PCR results. We also tested for translocat-

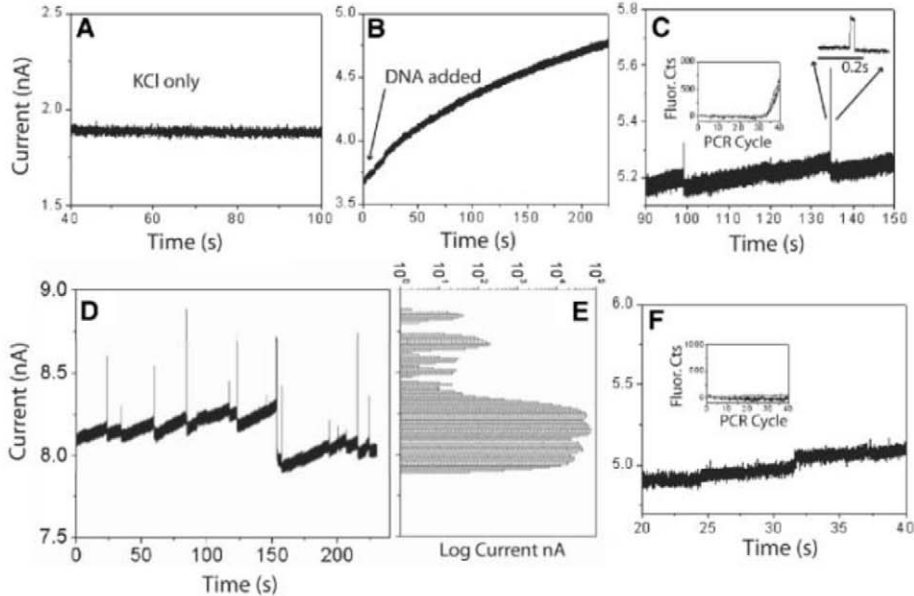


Fig. 3. Ion current signals of DNA translocation. (A) Current (2 M KCl, 1 mM PBS, pH 7) before DNA addition. (B) After DNA addition, current slowly increases. (C) 5 min after addition of 0.1-nM 60-nt DNA, large positive current spikes appear. These spikes are followed by a drop in baseline over a period of a second or so and then by a gradual rise leading to the next spike. (D) Representative data from another tube (also 60-nt DNA), with the distribution of currents shown in (E). The DNA causes large changes in baseline in addition to the spikes. (F) Data from a tube that showed both a current increase on DNA addition and baseline fluctuations but no spikes. No translocation was detected by PCR. The insets in (C) and (F) show the fluorescence signal from double-stranded DNA dye labels as a function of the PCR cycle number for samples collected from these particular runs.

Table 2. Results of QPCR tests for translocation in tubes with conductance >2 nS that gave uncontaminated control signals (data from four other devices that showed contamination in the control sample were rejected). Errors in spike count reflect the consequences of different cut-off criteria for selecting spikes. Errors in the molecule count were dominated by uncertainties in the filter recovery efficiency, except for the data marked *, which were calibrated with a second oligomer.

Tube	Tube conductance nS (1 M KCl)	DNA sample (nt)	Number of spikes	Number of molecules	Molecules per spike
AD1	9.7	60	350 ± 50	8000 ± 2000	23 ± 10
AD2	9.5	60	30 ± 10	400 ± 200	13 (+17, -13)
AA New1	19.6	120	64 ± 10	8500 ± 3100	88 (+126, -88)
AA New2	2.7	120	1500 ± 200	24,400 ± 5700	16 ± 7
HL-4-1-36	9.6	60	36 ± 4	1224 ± 774*	34 ± 21*
A136	1.6	60	46 ± 5	1900 ± 200*	41 ± 10*
HL-4-1-41	4.8	60	0	0	—
HL-4-1-40 O8	2.7	60	0	0	—

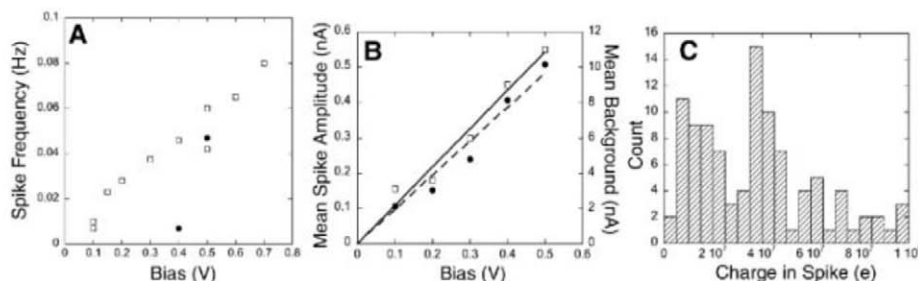


Fig. 4. Characteristics of the translocation signals for 60-nt DNA. **(A)** Spike rate increases with bias after a threshold that depends on the particular CNT; the two devices here show spike signals above 0.1 (squares) and 0.4 V (circles). **(B)** Spike amplitude (squares) increases linearly with bias and is about 5% of the background current (circles). **(C)** Distribution of the charge in each spike for the SWCNT in units of the electronic charge, e .

tion in “failed” control devices (i.e., lacking the CNT and deliberately overetched) that displayed leakage current. A few with very large leakage current showed evidence of DNA in the output well, but none displayed spikes, regardless of the magnitude of the leakage current. Thus, the spikes signal translocation of DNA through the SWCNTs.

QPCR also provides a measure of the number of molecules collected. We collected small samples of fluid from the output reservoir by flushing the system through with excess buffer and concentrated the solution using a Microcon YM-10 centrifugal filter so that we could redilute with PCR buffer. The filter losses were found to be highly variable, more so at low DNA concentrations, and account for much of the stated uncertainty in our results. We calibrated the PCR reaction with known amounts of DNA and, for two data points, calibrated filter losses by adding a known amount of a second sequence (with orthogonal primers) and carrying out a PCR analysis of both the target and calibration samples. The final molecule count was corrected for filter losses and dilution during the sample collection. The various errors in these steps tend to underestimate the amount of DNA that translocated, so the final results are probably lower limits. PCR was limited to the first use of a device, and we rejected samples from chips that showed contamination in the control samples collected.

We were able to carry out PCR on samples collected from 12 devices that had a conductance >2 nS. Of these, four had DNA contamination in control samples, leaving the eight devices listed in Table 2. Two of these showed no spikes and yielded no PCR signal. The remaining six all appeared to pass more than one molecule per spike. In particular, tubes HL-4-1-36 and A136, for which the filter recovery was directly measured with a control sample, passed at least 30 to 40 molecules for each spike. It is possible that the tube fills entirely with DNA, the spike signaling the cooperative emptying (or possibly filling) of the tube. The uncertainties in the PCR measurement are too large to reveal any significant difference between the

number of molecules per spike for the 60-nt sample (23, 13, 34, and 41) and the 120-nt sample (88 and 16), although the spike frequency was much lower in the two 120-nt runs, and the spike duration significantly longer (figs. S13 and S14).

Figure 4A shows data for the spiking rate as a function of bias for two different tubes passing 60-nt DNA. The spike rate increased with applied bias, and the two tubes showed different threshold biases for the onset of spikes (and hence translocation). For the 60-nt DNA, the spike amplitudes are about 5% of the baseline current (Fig. 4B), and their duration is between 3 and 100 ms, independent of applied bias, as long as it is above the threshold for translocation. The product of the spike duration and amplitude yields the charge contained in each spike (Fig. 4C). This is remarkably large, at about 1 pC or 10^7 electrons in each spike. Fan *et al.* explained positive charge spikes observed in nanochannels as a consequence of additional mobile ions brought into the channel by DNA molecules (13). Filling the tubes (2 μ m long) with 100 (20 nm long) 60-nt DNA oligomers, each carrying 60 excess electronic charges would account for only 1 part in 10,000 of the observed charge in each spike. The spikes must originate with large changes in the polarization outside the tubes, much as observed in junctions between micro- and nanochannels (28). The charge accumulation caused by the asymmetrical current in the SWCNT might be the source of this polarization, but further modeling is required to shed light on this unusual signal.

The excess ionic conductance appears to be a characteristic of metallic tubes, and we have proposed a mechanism based on electroosmotic flow resulting from trapped charge. Tubes with high ionic conductance will transport DNA molecules, giving a distinctive and unexpectedly large electrical signal of translocation. This kind of nanopore combines a long channel (in which translocation speed might be slowed) with an “integrated” electrode that might prove useful in new schemes for sequencing DNA by tunneling (3). The ability to select metallic SWCNTs of a desired diameter (29) may open the way

for production of devices with particular pore sizes.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5961/64/DC1

Materials and Methods

Figs. S1 to S14

Tables S1 to S3

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Solid Nanoparticles that Catalyze Biofuel Upgrade Reactions at the Water/Oil Interface

Steven Crossley, Jimmy Faria, Min Shen, Daniel E. Resasco*

A recoverable catalyst that simultaneously stabilizes emulsions would be highly advantageous in streamlining processes such as biomass refining, in which the immiscibility and thermal instability of crude products greatly complicates purification procedures. Here, we report a family of solid catalysts that can stabilize water-oil emulsions and catalyze reactions at the liquid/liquid interface. By depositing palladium onto carbon nanotube–inorganic oxide hybrid nanoparticles, we demonstrate biphasic hydrodeoxygenation and condensation catalysis in three substrate classes of interest in biomass refining. Microscopic characterization of the emulsions supports localization of the hybrid particles at the interface.

In phase-transfer catalysis, reactions are carried out in a biphasic mixture of two immiscible solvents, often water and a hydrophobic organic liquid; added surfactants such as quaternary ammonium salts enhance interfacial surface area (through emulsification) and facilitate transfers between the phases (1). The technique is especially useful in cases in which a product might otherwise be unstable under the reaction conditions in one phase but can partition into the other phase after rapid formation. More broadly, ongoing partitioning of by-products on the basis of their relative solubility can result in substantial simplifications at the isolation and purification stages, obviating the need for procedures such as distillation that might damage heat-sensitive compounds. Such process improvements could have a major impact in the field of biomass conversion to fuels. For example, bio-oil obtained from pyrolysis of biomass is a complex liquid that is only partially soluble in either water or hydrocarbon solvents (2). Rather than carrying out multiple consecutive purification steps during refining to separate out the hydrophilic by-products incompatible with fuel applications (3), it would be desirable to perform sequential reactions under phase-transfer conditions in a single reactor medium.

A serious drawback in such systems, however, is that the surfactants can be difficult to separate from final product mixtures. Solid particles are more easily recoverable and have also been shown in many cases to stabilize aqueous-organic emulsions (4–7), but these solid-stabilized emulsions have not been widely used in catalytic contexts. Moreover, in cases such as the refining of bio-oils in which the system is biphasic and contains up to 30% water, the most efficient way of catalyzing reactions is to place the solid catalyst at the liquid/liquid interface and to maximize the extent of interface by creating an emulsion. Otherwise,

the catalyst particles will preferentially remain in the heavier phase, such as water. In that case, only the water-soluble molecules will be converted. If further conversion of water-insoluble molecules is wanted, one would need to remove them from the top of the reactor and send them to another reactor with a catalyst operating in the organic phase. Therefore, the concept of solid particles that can simultaneously stabilize an emulsion and catalyze reactions in both phases becomes an attractive proposition.

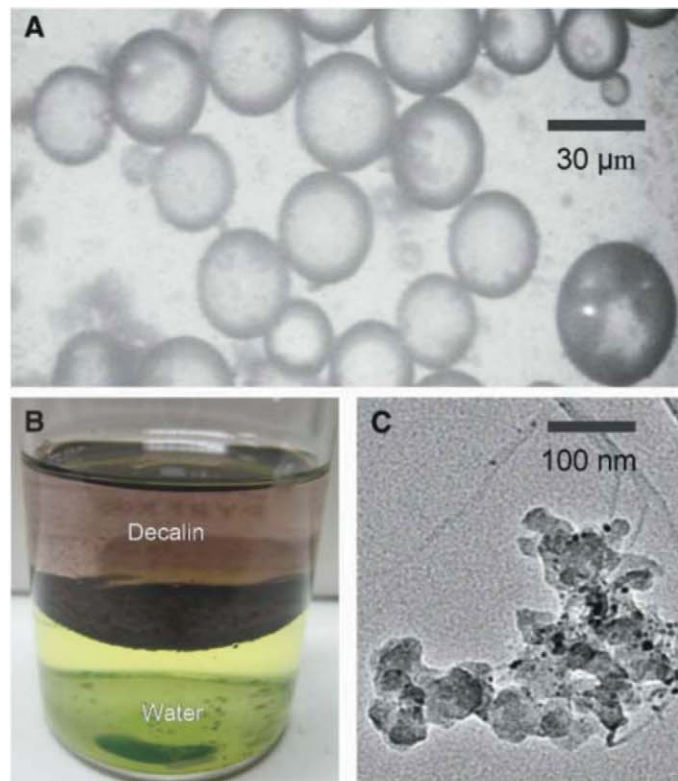
In particular, oxide nanoparticles have previously been used to stabilize oil-in-water emulsions (8–10) because their hydrophilicity preferentially orients them toward the aqueous phase at the inter-

face. Carbon nanotubes have also been shown to produce emulsions, but of the water-in-oil variety because they are hydrophobic (11). We recently prepared hybrid nanoparticles by fusing carbon nanotubes to silica (12). By tuning their composition, we could modify the hydrophilic-hydrophobic balance and assemble water-in-oil or oil-in-water emulsions systematically and reproducibly (13). The emulsion volume fraction in the three-layer system formed (oil-emulsion-water) and the droplet size were greatly influenced by the oil-to-water ratio in the mixture and the degree of oxidative functionalization of the nanotubes. In this contribution, we have undertaken a more detailed characterization of the structure of these emulsions using optical and electron microscopy [figs. S14 to S16 and supporting online material (SOM) text] (14).

One of the main objectives of the present study was to extend the utility of these nanohybrids by incorporating a transition metal, rendering them catalytically active for hydrogenation. The second objective was to add a solid base function to catalyze condensation reactions.

As shown in Fig. 1, after the addition of Pd metal particles these particles still straddle the organic/aqueous interface, and the appearance and stability of the emulsions formed are unaffected. We envisioned that their selective metal functionalization would be a powerful strategy for directing reactivity in a specific phase: Depositing a metal such as Pd on the hydrophilic face would catalyze aqueous reactions, whereas deposition on the hydrophobic face would favor chemistry in the organic solvent. In fact, established catalyst

Fig. 1. (A) Optical microscopy image of a water-in-oil emulsion formed by sonicating a 1:1 mixture of decalin and water in the presence of 5 weight percent (wt %) Pd/SWNT-SiO₂ nanohybrids. (B) The same mixture as in (A) before sonication. It is seen that the nanohybrids preferentially migrate to the interface. (C) TEM image of the 5 wt % Pd/SWNT-SiO₂ nanohybrids. It is apparent that Pd clusters are preferentially located over the silica surface rather than over the nanotubes.



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preparation methods exist that can be used to selectively deposit metal precursors from solution on either silica or nanotubes, depending on the pH of the solution and the point of zero charge of the substrate (15–17).

In this work, we have explored two preparations with nanotubes of different type. This difference affects the deposition of Pd. As shown in Fig. 1C, in the first preparation the incipient wetness impregnation method used to deposit Pd on the single-walled carbon nanotube (SWNT)–silica nanohybrids results in preferential deposition onto the silica side. This preferential deposition is

due to the very low density of defects on the SWNT walls, as demonstrated by means of transmission electron microscopy (TEM) and Raman spectroscopy (fig. S1). Without defects, the SWNT cannot effectively anchor the Pd nanoparticles, which during the heat treatments end up on the silica surface. In contrast, on the second preparation type, which involves MgO as a support instead of SiO₂, the resulting nanotubes are more defective than those in the first preparation, as also shown by means of TEM and Raman spectroscopy (fig. S2). Consequently, they are more effective in retaining Pd particles. Therefore, we

have been able to demonstrate different degrees of hydrogenation activity in the organic phase by simply using different formulations of the nanohybrids.

We present here the results obtained for several reactions of relevance to biomass-refining chemistry. Two of the major goals in the refining of liquids derived from lignocellulosic sources are the elimination of oxygen and the condensation of small molecules. The former is needed to improve the low stability caused by the high reactivity of the oxygenated functional groups in molecules such as the phenolic compounds de-

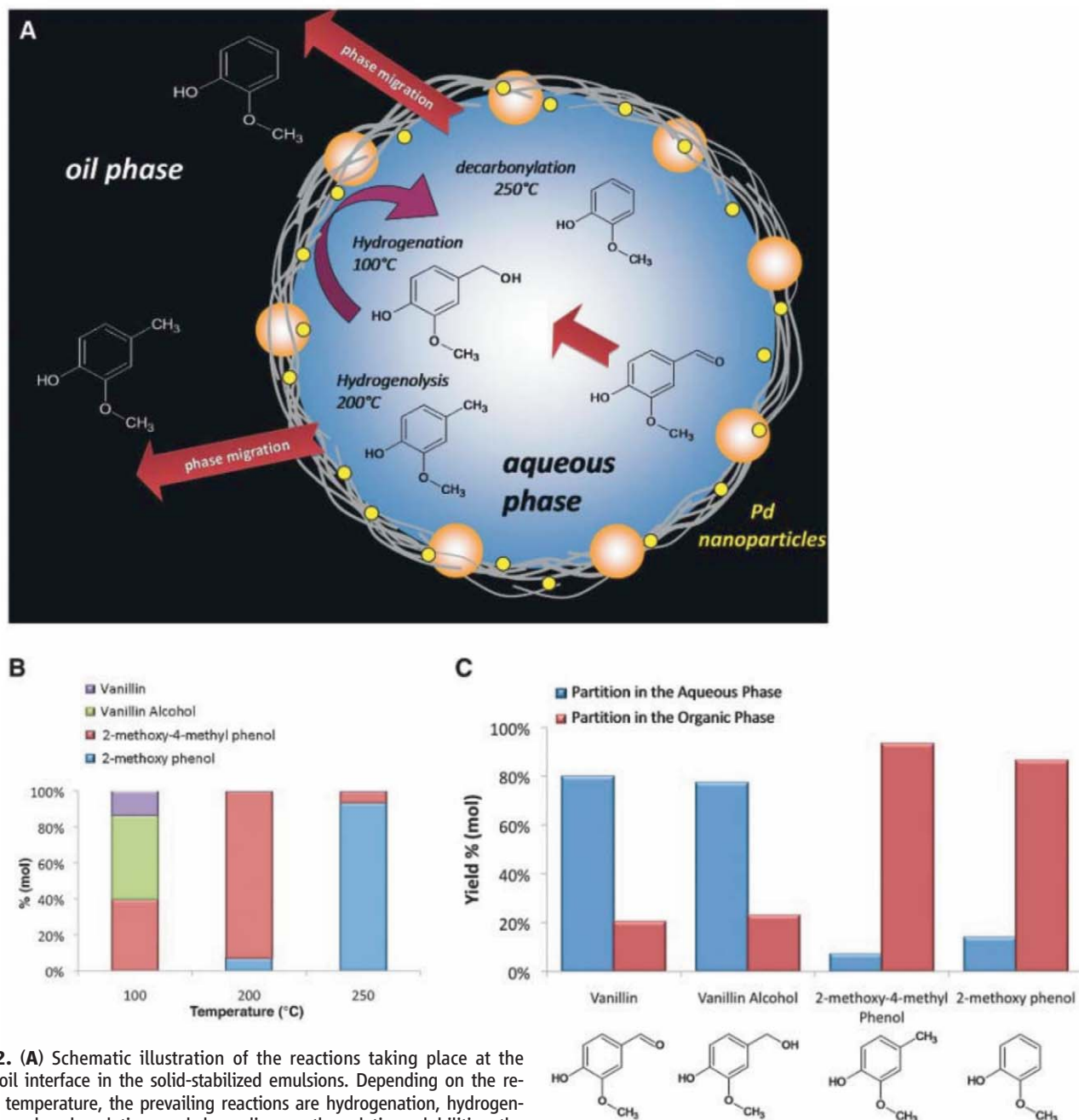


Fig. 2. (A) Schematic illustration of the reactions taking place at the water/oil interface in the solid-stabilized emulsions. Depending on the reaction temperature, the prevailing reactions are hydrogenation, hydrogenolysis, or decarbonylation, and depending on the relative solubilities, the products remain in the aqueous phase or migrate to the oil phase. (B) Total weight fraction of the various products as a function of temperature after 30-min reaction in a batch reactor, from gas chromatographic analysis of each phase (combined). (C) Partition of the various products as in (B) between the individual aqueous and organic phases.

rived from lignin (18, 19). The latter is particularly important to increase the molecular weight of those light fragments derived from the less refractory parts of the biomass (cellulose and hemicellulose).

To illustrate the application of the catalytic nanohybrids in emulsions, we chose the hydrodeoxygenation of a phenolic compound and the hydrogenation and etherification of an aldehyde on Pd as model reactions that occur in the aqueous side of the interface. Therefore, we have used the nanohybrids that preferentially have Pd on the hydrophilic side.

In addition, we have been able to replace silica as the hydrophilic component in the nanohybrids with a basic oxide, magnesite, which not only is effective in stabilizing emulsions but also provides catalytic activity for base-catalyzed reactions, such as the Aldol condensation. In this case, as mentioned above, the hydrophobic side contains nanotubes that are more defective, which allow stabilization of Pd. Therefore, hydrogenation on the oil side is readily observed.

To explore the catalytic application of Pd-containing nanohybrids to phenolic hydrodeoxygenation in a water-in-decalin emulsion, we chose vanillin (4-hydroxy-3-methoxybenzaldehyde) as a test substrate because it is a common component of pyrolysis oil derived from the lignin fraction (20, 21). This compound was appealing for study because of its three different types of oxygenated functional groups (aldehyde, ether, and hydroxyl) and its partial solubility in both the organic and aqueous phases.

The reaction was carried out in a batch reactor at three different temperatures (100, 200, and 250°C) for different reaction periods. In each run, the molar ratio of vanillin to Pd in the reactor was about 100. After each reaction period, the emulsion was broken by filtering out the nanohybrid particles. The filtering was conducted in two steps. In the first one, a common paper filter was used. This coarse filter (8- μm pores) was able to trap a large fraction of nanohybrids because they quickly agglomerate when they contact the filter. In the second step, a polytetrafluoroethylene (0.2- μm pores) filter was used to separate the small fraction of nanohybrids that passed the first filter. The two liquid phases were separated and analyzed individually by means of gas chromatography with flame ionization (FID) and mass spectrometry (MS) detectors. It was then possible to monitor the migration that the different products underwent from the aqueous to the organic phase, as summarized in Fig. 2. Whereas vanillin and vanillin alcohol were highly soluble in the aqueous phase, the products resulting from both hydrogenolysis and decarbonylation were much more soluble in the decalin phase. As a result, the water-insoluble compounds, which are more valuable as fuel components, can be readily incorporated in the product stream.

The turnover number measured at the lowest temperature tested (100°C) was about 0.2 s^{-1} , which is of similar magnitude to that observed by

others in the hydrogenation of guaiacol catalyzed by Pd/C in a monophasic system but at higher temperatures (150°C) (19). The higher activity observed in the biphasic system could be ascribed to (i) a better state of particle dispersion at the interface as compared with that in the monophasic system (fig. S13) and/or (ii) enhanced hydrogen concentration at the interface (hydrogen has higher solubility in organic phase than in water).

As illustrated schematically in Fig. 2A, we observed a range of different products and phase-migration processes that were due to the varying extents of hydrogenation, hydrogenolysis, and decarbonylation reactions catalyzed by Pd as the reaction conditions were modified. As shown in Fig. 2B, the chemoselectivity changes significantly with increasing temperature. At 100°C, initially only hydrogenation of the aldehyde to the vanillin alcohol is observed, whereas at longer reaction times the alcohol is further converted into 2-methoxy-4-methylphenol (p-cresol) via hydrogenolysis. At 200°C, hydrogenolysis becomes the dominant path even at short reaction times. At 250°C, the dominant reaction is the decarbonylation of the aldehyde group, leading primarily to o-methoxyphenol (guaiacol).

The variation of product distribution with reaction time in the batch reactor at 100°C is shown in fig. S5. The vanillin alcohol is the primary product, but at longer times it is consumed by hydrogenolysis to form p-cresol, which migrates to the organic phase upon formation, preventing further conversion. In contrast, the alcohol remains in the aqueous phase and continues reacting. This result illustrates the concept of simultaneous reaction and separation of intermediate products.

We next explored the reactivity of molecules that were exclusively soluble in either the organic or aqueous phase. Octanal and glutaraldehyde were chosen as the model compounds soluble in

the decalin and water phases, respectively. Three reaction runs were compared at the same conditions (14). In one of them, octanal and glutaraldehyde were partitioned in an equimolar mixture of water and decalin. In the other two, octanal and glutaraldehyde were separately dissolved in pure decalin and pure water, respectively. These molecules were readily hydrogenated over Pd to the corresponding alcohols. However, the nanohybrids that were used in this case had the metal preferentially deposited on the hydrophilic side. Therefore, we anticipated to see a greater effect on the conversion of glutaraldehyde. Indeed, although only 58% conversion was obtained in the single aqueous phase after 3 hours at 100°C, practically all (98%) of the glutaraldehyde was converted in the emulsion under the same conditions. This enhancement can be ascribed to a combination of selective deposition of Pd on the polar silica as well as the increased exposure of the catalyst at the interface, as compared with the highly aggregated state of the solid catalyst suspended in the one-phase system. Although 5-hydroxypentanal was the expected primary product from the initial hydrogenation of one of the carbonyl groups in glutaraldehyde, this product was not observed. Instead, the cyclic hemiacetal, valerolactol, emerged as a major product. We suspect that the cyclization of the hydroxyaldehyde occurs via attack by the nucleophilic oxygen of the alcohol at the carbonyl carbon, which is analogous to the well-known cyclization of glucose (22).

The time evolution of the products (fig. S6) indicates that the reaction sequence is glutaraldehyde $\rightarrow$ δ -valerolactol $\rightarrow$ 1,5 pentanediol, with the diol dominating at long reaction times. An additional product, 5-hydroxy-1-pentyl tetrahydropyranyl ether, was also observed, but in low yield (<2%). Whereas glutaraldehyde, octa-

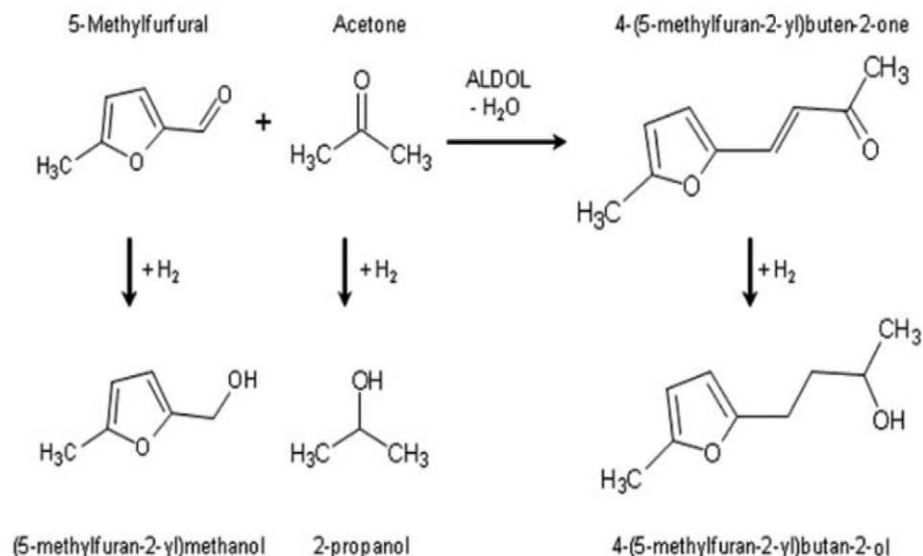


Fig. 3. Possible reaction paths and products arising from the condensation and direct hydrogenation of 5-methylfurfural and acetone over 5 wt % Pd/CNT/MgO.

nediol, and valerolactol remained in the aqueous phase, the ether migrated to the decalin phase. This is an example that illustrates the potential of the method by which one can easily separate an intermediate product from the reaction mixture—even when it is obtained at low yield—before it continues reacting.

At higher temperature, the ether underwent decarbonylation or hydrogenolysis before leaving the aqueous phase, producing 2-butoxytetrahydro-2H-pyran or 2-pentyloxytetrahydro-2H-pyran, respectively. In good agreement with the very high log *P* values of these two products, they were observed exclusively in the oil phase, once again demonstrating the concept of separation after reaction. This result shows that one could arbitrarily modify selectivity by controlling the relative rates of reaction and migration out of the phase in which the catalyst is located. Analogous to shape-selectivity in zeolites (23) and selectivity affected by mass transfer phenomena (24), we can introduce the concept of a phase-transfer selectivity, by which the product distribution resulting from reactions catalyzed at the liquid/liquid interface is modified by the transport to and through the interface, where the catalyst resides. One could design emulsion systems with varying character-

istics (such as droplet size, emulsion type, and relative solubilities) that may affect the residence time of intermediate products in one phase before it migrates to the other phase. This controlled migration may in turn affect the extent of reaction of one specific intermediate.

Because very little Pd was present on the hydrophobic side, in the case of the octanal reaction we observed the opposing result: an activity enhancement in the pure organic phase relative to the emulsion. The octanol yield on the single phase was 9.1%, whereas that in the emulsion was only 2.3%. We ascribe this outcome to the larger proportion of Pd deposited on the hydrophilic oxide than on the hydrophobic nanotubes, as shown in Fig. 1C by means of TEM. The direct hydrogenation of octanal in the organic phase yielded 1-octanol as the (expected) dominant product, together with a small quantity of the symmetric dioctyl ether derived from alcohol coupling.

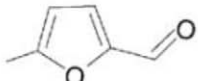
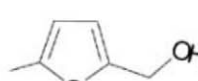
Finally, we explored a tandem reaction sequence in which Pd-catalyzed hydrogenation was paired with a preceding Aldol condensation of 5-methylfurfural and acetone. This reaction was catalyzed by MgO, which was incorporated in the nanohybrids instead of silica. When using

MgO during the nanotube synthesis, the SWNT product contains about 20% multiwalled carbon nanotubes (MWNTs) as an impurity; however, the use of MgO imparts basicity to the nanohybrids, which is crucial for the Aldol condensation. Therefore, because the MgO-based nanohybrids are still effective in stabilizing emulsions, we have been able to conduct reactions at the liquid/liquid interface by using a bifunctional catalyst that contains both metal and basic sites. Moreover, as mentioned above, this nanohybrid contains nanotubes that are more defective and is able to stabilize Pd particles not only on the hydrophilic side but also on the hydrophobic side. Therefore, hydrogenation can occur on both sides of the emulsion.

As represented in Fig. 3, the expected products from the combination of these reactions are 4-(5-methylfuran-2-yl)buten-2-one, 4-(5-methylfuran-2-yl)buten-2-ol, 5-methylfuran-2-yl methanol, and 2-propanol. To better determine how these products evolve, we conducted this reaction in tandem. That is, we first ran the reaction under N₂ at 80°C for 3 hours and analyzed the products, which is indicated as reaction 1 in Table 1. In this case, no hydrogenation took place. Self-condensation of acetone was not observed. Ketone-ketone condensation reactions are thermodynamically less favorable and much slower than ketone-aldehyde condensation, as pointed out by West *et al.* (25). As a result, the major product was 4-(5-methylfuran-2-yl)buten-2-one, which in line with its high log *P* value (~1.5) migrated almost completely to the organic phase. In the second experiment, which is indicated as reaction 2 in Table 1, an additional 1-hour reaction step at 100°C under H₂ flow was added to the initial 3 hours at 80°C under N₂. The 4-(5-methylfuran-2-yl)buten-2-one is totally hydrogenated in the second step, indicating that hydrogenation has occurred in the organic phase as well as in the aqueous phase.

The shorter oxygenates, in this case propanol, remain in the aqueous phase, thus enhancing the probability of realizing further condensation reactions that would be advantageous in the production of fuel components. Similar reactions have been previously described by Dumesic and co-workers (26, 27), operating with a monophasic system. The advantage of operating in a biphasic system, with the catalyst at the liquid/liquid interface, is the possibility of conducting the sequential reactions in a single reactor instead of two. The carbon chains migrate to the organic phase after growing long enough to become hydrophobic, facilitating their isolation as desirable products, whereas the shorter chains remained in the aqueous phase to undergo further growth. The use of a biphasic system provides the possibility of doing the next reaction (for example, hydrogenation-hydrogenolysis exclusively in the oil phase) but in the same reactor. In previous studies conducted in a monophasic aqueous system (26), once the water solubility of the condensed product becomes low enough, it leaves this phase, stopping the conversion. In many cases, these intermediate products

Table 1. Product distribution of the Aldol-condensation–hydrogenation reaction of 5-methylfurfural and acetone over 5 wt% Pd/(CNT/MgO). Reaction conditions were 3 hours at 80°C in 250 pounds per square inch (psi) of N₂ and then 1 hour at 100°C in 250 psi of H₂. Aq., aqueous phase; Org., organic phase.

wt % in each phase → ↓ Compound	Aq.	Org.	Aq.	Org.	Aq.	Org.
	Feed		Reaction 1		Reaction 2	
	92.5	60.8	91.7	56.0	85.8	56.7
	0.0	0.0	0.0	0.0	4.0	0.0
	7.5	39.2	6.3	31.5	4.1	24.6
	0.0	0.0	0.0	0.0	3.6	2.3
	0.0	0.0	2.0	12.5	0.0	0.0
	0.0	0.0	0.0	0.0	2.5	16.4
Total in each phase	100	100	100	100	100	100

still contain oxygen, and the only way to completely deoxygenate them is by using a second reactor operating in the organic phase or a high-temperature vapor phase hydrotreatment.

With solid-stabilized emulsions, a continuous process could be designed in which the two homogeneous phases coexist with the emulsion in a layered configuration: oil/emulsion/water. One can achieve full conversion on both sides of the emulsion followed by constant removal of oil-soluble products from the top layer and water-soluble products from the bottom layer while the reaction keeps occurring in the emulsion.

Our results highlight the preliminary applications of solid catalysts localized at the interface between two liquid phases. We anticipate that tailoring such emulsion-stabilizing solids with additional catalytic functional groups will facilitate a broad range of reactions.

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Unveiling the Transient Template in the Self-Assembly of a Molecular Oxide Nanowheel

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Self-assembly has proven a powerful means of preparing structurally intricate nanomaterials, but the mechanism is often masked by the common one-pot mixing procedure. We employed a flow system to study the steps underlying assembly of a previously characterized molybdenum oxide wheel 3.6 nanometers in diameter. We observed crystallization of an intermediate structure in which a central {Mo₃₆} cluster appears to template the assembly of the surrounding {Mo₁₅₀} wheel. The transient nature of the template is demonstrated by its ejection after the wheel is reduced to its final electronic state. The template's role in the self-assembly mechanism is further confirmed by the deliberate addition of the template to the reaction mixture, which greatly accelerates the assembly time of the {Mo₁₅₀} wheel and increases the yield.

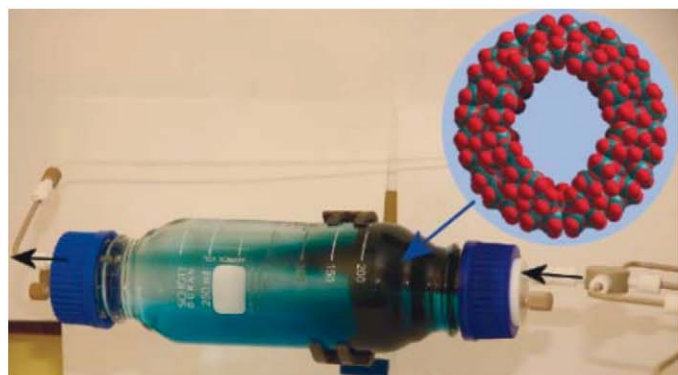
The bottom-up self-assembly of large inorganic architectures is a key synthetic route for the preparation of a whole range of systems from cages (1–3) to metal organic frameworks (4, 5) and the formation of macrocycles (6) from comparatively simple small molecule building blocks. In these systems the building blocks, and the underlying self-assembly processes, are understood to such a high degree that many complex and intricate structures can be designed from first principles, and the resulting architectures can even be

postsynthetically modified (7). However, when nanostructures in the 2- to 10-nm range are targeted (e.g., metallic nanoparticles and quantum

dots), the self-assembly can be critically limited by the high number of degrees of freedom, and it is not trivial to target narrow size distributions. In contrast, in supramolecular chemistry, molecular templates have been successfully employed as external directors in the design of receptors (3, 8–10) and can facilitate the assembly of molecular nanostructures that are intrinsically monodisperse. Spectacular assembly control has been demonstrated by deliberate targeting and synthesis of templates that by design enable the formation of a predetermined structure, yet it is difficult to design large structures (2). The discovery of a similar templating strategy for the reliable fabrication of 2- to 10-nm molecular nanoparticles would revolutionize the synthesis and applications of molecular materials in the same way that templated synthesis has revolutionized the field of organic macrocyclic synthesis over the past 40 years.

In recent years, Müller and co-workers reported the solution-phase assembly of a family

Fig. 1. A photograph of the flow-reactor system showing the blue reduction wavefront gradient formed within the vessel during the assembly of compound **1** (the reduced region is blue, and the more oxidized region is pale to clear). The structure of the {Mo₁₅₀} wheel present in compound **1** is shown in space-filling ball-and-stick mode. Mo ions are green spheres; O ligands are red spheres.



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of nanoscale metal-oxide rings composed of 140 to 154 molybdenum centers by reduction of an acidic (pH = 1 to 3) molybdate solution, which have a range of interesting physical and chemical properties arising from their molecular nature, nanoscale size, and electronic distribution (11–15). These rings crystallized either as ordered chain/layer assemblies or discrete molecules. Diffraction analysis revealed that each individual ring appeared to have assembled from several classes of discrete $[\text{Mo}_x\text{O}_y]$ building blocks, most commonly $\{\text{Mo}_2\}$, $\{\text{Mo}\}$, and $\{\text{Mo}_8\}$ bonded together through oxo-bridges to form $\{\text{Mo}_{154-x}\}$ ($x = 0$ to 14) rings 3.6 nm in diameter. We hypothesized that some sort of internal templating must have played a role in such an intricate assembly process, but the deceptively simple reaction conditions have so far effectively concealed the enormous complexity involved in this self-assembly process. We probed the self-assembly of the molybdenum blue (MB) nanoparticles using a dynamic synthetic procedure in a flow system that enabled real-time adjustment of the three input variables (pH, concentration of molybdate, and reducing agent) controlling the synthesis of the molecular nanosized-wheels (Fig. 1).

By using a flow system, rather than combining all reagents at once in a single flask (“one pot”), we were able to maintain an off-equilibrium reaction system in which we carefully controlled the degree of reduction of the polyoxomolybdate clusters. To achieve this reaction state, screening

of the synthetic parameters—for example, the concentration of molybdate, reducing agent, pH, and ionic strength—was required to determine the correct flow rate. If the flow rate was too low, then the reduced molybdate would be produced in such low concentration that crystallization would not occur and the reduced molybdate within the system would be reoxidized by oxygen over a period of days (i.e., the solution would become colorless). If the flow rate was too high, then the whole system would become over-reduced and no gradient between the reduced and oxidized regions would be set up on a time scale that would allow crystallization (i.e., the solution would become uniformly dark blue).

However, after optimizing the flow system, we were also able to isolate and trap, by crystallization, a key intermediate in the assembly of wheel-type MB nanocluster whereby single crystals were formed in the flow reactor and isolated by halting the flow and filtering the product after a given run time of 2 to 3 days. Specifically, we characterized a hollow $\{\text{Mo}_{150}\}$ wheel that hosts a $\{\text{Mo}_{36}\}$ cluster that is bound to the central cavity of the ring species by charge-balancing sodium cations, and in the solid state the wheel itself is weakly covalently linked through 5 oxo-bridges to chains (16). This host-guest complex shows features indicative of an intermediate electronic and structural state, and we postulate that the $\{\text{Mo}_{36}\}$ cluster acts as the key template in the formation of the MB ring. We isolated the host-guest complex as the crystalline compound **1**, $\text{Na}_{22}[\text{Mo}_{36}^{\text{VI}}\text{O}_{112}(\text{H}_2\text{O})_{16}] \subset [\text{Mo}_{130}^{\text{VI}}\text{Mo}_{20}^{\text{V}}\text{O}_{442}(\text{OH})_{10}(\text{H}_2\text{O})_{61}] \cdot 180\text{H}_2\text{O} \equiv \text{Na}_{22}\text{-}\mathbf{1a} \cdot 180\text{H}_2\text{O}$ in a gram yield of 4.5 g, 17.4% by the reduction of an aqueous acidic solution of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ with $\text{Na}_2\text{S}_2\text{O}_4$ under continuous addition of HNO_3 , and we were able to determine the formula of the host-guest compound

unambiguously using many lines of investigation (16). The key to this discovery was the use of nitric acid in the flow system in a dual role as a proton source and an oxidant leading to incomplete reduction of the wheel. The archetypal wheel $\{\text{Mo}_{154}\}$ has 14 two-electron reduced compartments (i.e., a total of 28 4d electrons), but in the present case, only 10 of the 14 compartments are two-electron reduced, rendering the overall intermediate wheel 20-electron reduced (as confirmed by a combination of structural studies, chemical analysis, redox titration, and solution UV-VIS spectroscopy). Compound **1** crystallizes in the space group $C2/m$ and has a large unit cell with a volume of $41,734 \text{ \AA}^3$. The corresponding formula was determined by elemental analyses, single-crystal x-ray structure analysis, bond valence sum calculations, redox titrations, and thermogravimetry (16). Structural analysis revealed that the $\{\text{Mo}_{150}\}$ wheel features an ellipsoidal central cavity of $\sim 2.6 \times 1.8 \text{ nm}$ in which a $\{\text{Mo}_{36}\}$ unit resides. The principal axis of the $\{\text{Mo}_{36}\}$ template is tilted by 38.5° with respect to the main wheel plane and is linked to the $\{\text{Mo}_{150}\}$ wheel through sodium cation bridges that reduce the electrostatic repulsion between the two negatively charged constituents (Fig. 2), and the binding of the guest is also stabilized by hydrogen-bonded interactions to the host. The $\{\text{Mo}_{150}\}$ wheel in **1a** consists of 14 $\{\text{Mo}_8\}$ -type pentagonal building units (blue, Fig. 2) which are linked along the inner rim of the wheel by 12 $\{\text{Mo}_2\}$ linker units (red, Fig. 2), as opposed to the 14 $\{\text{Mo}_2\}$ units present in the archetypal wheel (4, 15), and connected by 14 $\{\text{Mo}_1\}$ groups along the central equatorial region of the structure (yellow, Fig. 2), so that the wheel-type cluster in **1a** can be formulated as $\{\text{Mo}_{150}\} = (\{\text{Mo}_1\}_{14}\{\text{Mo}_2\}_{12}\{\text{Mo}_8\}_{14})$; the decrease in the number of the $\{\text{Mo}_2\}$ building blocks in MB



Fig. 2. A polyhedral representation of the nanoscale $\{\text{Mo}_{36}\} \subset \{\text{Mo}_{150}\}$ wheel as part of the chain **1a** [for linking details, see fig. S3 and the chains with pure wheels in (19)]. Sodium cations have been omitted for clarity, although there are ~ 22 positions on the inner side of the ring where sodium resides, of which 12 directly bridge the template to the outer ring. The host and the guest are also connected by a series of hydrogen bonds. The polyhedral building blocks are colored as follows: $\{\text{Mo}_1\}$, yellow; $\{\text{Mo}_2\}$, red; $\{\text{Mo}_8\}$, blue with a light blue pentagonal central group.

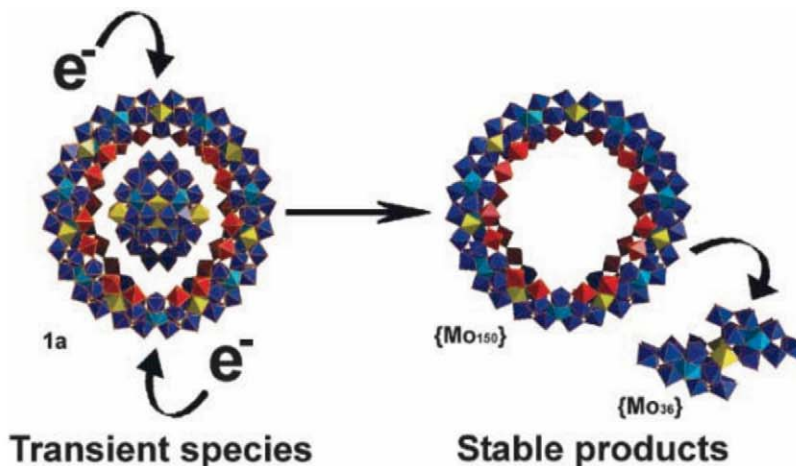


Fig. 3. Representation of the expulsion process. The addition of eight additional electrons increases the repulsion between the ring and the template, leading to the expulsion of the $\{\text{Mo}_{36}\}$ unit, that is, a separation into a pure $\{\text{Mo}_{150}\}$ phase and a pure $\{\text{Mo}_{36}\}$ phase (both isolated as crystalline solids). Color scheme as in Fig. 2.

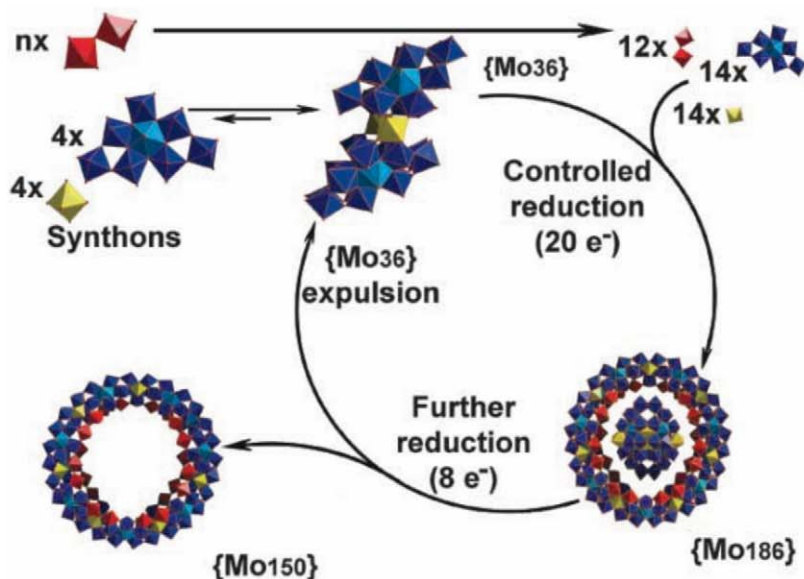


Fig. 4. Conceptual representation of the MB assembly showing the building block “synthons” (which are assigned on the basis of structural considerations) that form the template complex. The $\{Mo_{36}\}$, $\{Mo_{150}\}$, and $\{Mo_{36} \subset Mo_{150}\}$ complexes have each been isolated separately. Continuous flow-reaction conditions, along with a finely tuned reducing environment, are required to trap the template complex. Color scheme as in Fig. 2.

wheels has been previously documented as the formation of “defect” sites (17–19). The $\{Mo_{150}\}$ wheel in **1a** can be geometrically related to the ring-shaped $\{Mo_{154}\} \equiv [Mo_{154}O_{462}H_{14}(H_2O)_{70}]^{14-}$ cluster archetype (11,14). According to the classification devised to describe these MB ring-type architectures (14, 15, 17), the formula of the $\{Mo_{150}\}$ wheel in **1a** can be approximately expressed in terms of the building blocks $[Mo_2]_{12}[Mo_8]_{14}[Mo_1]_{14}]^{14-}$, and the overall formula is $[Mo_{130}^{VI}Mo_{20}^{V}O_{442}(OH)_{10}(H_2O)_6]^{14-}$ (20). The formula was deduced with the help of bond valence sum analysis on the structural data (distinguishing between the reduced and non-reduced Mo centers and between protonated and nonprotonated O atoms), and this was confirmed chemically using redox titrations and chemical analysis [see (16) for a detailed analysis of the wheel structure].

The transient nature of the wheel-template complex is supported by comparative reactivity studies and is reflected in its geometric and electronic structure. In contrast to the archetypal, highly symmetrical D_{7d} $\{Mo_{154}\}$ MB wheel, the $\{Mo_{150}\}$ wheel in **1a** is elliptical with maximum outer and inner ring diameters of ~3.6 and 2.6 nm, and minimum outer and inner ring diameters of ~3.5 and 1.8 nm (Fig. 3). This ellipsoidal structure appears to be a result of the central $\{Mo_{36}\}$ template: Comparison of the ratio between the maximum and minimum cluster dimensions for both the wheel and the template ($R_{1/2}$) shows a close match between $\{Mo_{36}\}$ ($R_{1/2} = 1.42$) and the inner ring of $\{Mo_{150}\}$ ($R_{1/2} = 1.44$). Another striking feature of the $\{Mo_{36}\} \subset \{Mo_{150}\}$ template-wheel as-

sembly is the nonuniform delocalization of 20 4d electrons over the molybdenum centers. Bond valence sum calculations (16) for the Mo centers of the $\{Mo_{150}\}$ wheel show that the Mo centers close to the two defect sites at the most compressed sections of the wheel are fully oxidized (+6), whereas those in the least compressed region are reduced to +5. This electronic anisotropy stands in contrast to the 28-electron reduced $\{Mo_{154-x}\}$ rings, where the electrons are delocalized more symmetrically over the cluster surface (14, 15, 18). The intermediate 20-electron reduced MB wheel clearly favors the inclusion of the anionic $\{Mo_{36}\}$ template more than the 28-electron reduced $\{Mo_{154-x}\}$ systems. This observation is confirmed by its further reduction in solution upon replacing nitric acid with HCl in the flow reactor, which results in the expulsion of the template and the crystallization of two separate crystalline phases, including the empty, fully symmetric, and 28-fold reduced $\{Mo_{150}\}$ wheel and the $\{Mo_{36}\}$ template (Fig. 3).

On the basis of these data, we postulate that the overall mechanism underpinning the formation of the $\{Mo_{154-x}\}$ family involves the $\{Mo_{36}\}$ cluster as a structure-directing template. In keeping with this hypothesis, the $\{Mo_{36}\}$ cluster is well known to form spontaneously in acidified molybdate solutions in the absence of reducing agent. As a result, we can formulate the mechanism (Fig. 4).

To test this hypothesis, we compared the time necessary to synthesize the wheel nanoparticles under static conditions, where the molybdate, reducing agent, and acid were added simulta-

neously, versus flow conditions in which $\{Mo_{36}\}$ was added to a reduced molybdate solution. In the latter case, crystallization started after only 6 to 8 hours, yielding gram quantities of the wheel-based MB nanoparticles within 1 day. The static system required between 3 and 5 days (after crystallization commenced) to produce the same amount of material as isolated from the flow system over the period of 1 day.

Our results illustrate how a bottom-up assembly process can be used to rapidly obtain gram quantities of a nanomaterial with well-defined size, shape, and composition. Furthermore, the use of a flow reactor proved to be a powerful tool in unveiling the mechanism of assembly of the $\{Mo_{154-x}\}$ nanowheel family, and we envision the technique emerging as a versatile means of generating off-equilibrium reaction conditions for mechanistic studies of self-assembly processes.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5961/72/DC1
Materials and Methods
Figs. S1 to S5
References
Movie S1

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Synchronous Deglacial Overturning and Water Mass Source Changes

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Understanding changes in ocean circulation during the last deglaciation is crucial to unraveling the dynamics of glacial-interglacial and millennial climate shifts. We used neodymium isotope measurements on postdepositional iron-manganese oxide coatings precipitated on planktonic foraminifera to reconstruct changes in the bottom water source of the deep western North Atlantic at the Bermuda Rise. Comparison of our deep water source record with overturning strength proxies shows that both the deep water mass source and the overturning rate shifted rapidly and synchronously during the last deglacial transition. In contrast, any freshwater perturbation caused by Heinrich event 1 could have only affected shallow overturning. These findings show how changes in upper-ocean overturning associated with millennial-scale events differ from those associated with whole-ocean deglacial climate events.

Changes in ocean overturning affect regional climate through the redistribution of heat energy to the high latitudes (1). Reconstructions of past ocean circulation changes suggest a strong link between the strength of Atlantic Meridional Overturning Circulation (AMOC) and circum-North Atlantic temper-

atures, as evidenced by the close match between rapid shifts in nutrient proxy records (2) and Greenland ice core paleotemperature records (3). Models of thermohaline circulation have suggested rapid changes in overturning strength and water mass stratification during meltwater-forced events, but also suggest that rate of overturning can be decoupled from stratification changes. However, changes in these primary physical variables cannot be unambiguously reconstructed through time by means of nutrient proxies of ocean circulation, because their sensitivity to source, circulation, and biological change complicates interpretation. This study directly compares water mass source changes, reconstructed

using Nd isotopes, with $^{231}\text{Pa}/^{230}\text{Th}_0$ ratios recording overturning rates at the same site.

Nd isotopes can be used as a water mass source proxy because (i) the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio (ϵ_{Nd}) is a tracer of deep water masses originating in different ocean basins (4, 5); (ii) the 500- to 1000-year residence time of Nd in the ocean (6) is shorter than the time required for whole-ocean mixing; and (iii) Nd isotopes are not fractionated by biological or low-temperature processes (7). The ϵ_{Nd} value for North Atlantic Deep Water (NADW) is ~ -14 , and for Antarctic Bottom Water (AABW) it is ~ -7 to -9 (4, 5, 7), at modern deep water formation sites. Nd isotopes can be used to track these water masses over long path lengths (7). In marginal settings with high suspended particle concentrations, the water mass ϵ_{Nd} appears to be altered by exchange with sedimentary Nd (8). Our site on the northeast Bermuda Rise (Fig. 1) is in the open ocean, oceanographically distant from major continental boundaries, and not located downstream of Bermuda. Although sediment deposition rates are high, the particles are primarily transported by recirculating gyres and any leachable material is predominantly authigenic (9). Application of ϵ_{Nd} together with other oceanographic proxies provides accurate reconstructions of ocean circulation and changes in climate.

Recent millennial-scale Nd isotope studies have used Fe-Mn oxides leached from bulk detrital sediment (10). However, horizontal advection of fine sediments (11) can transport distal Nd

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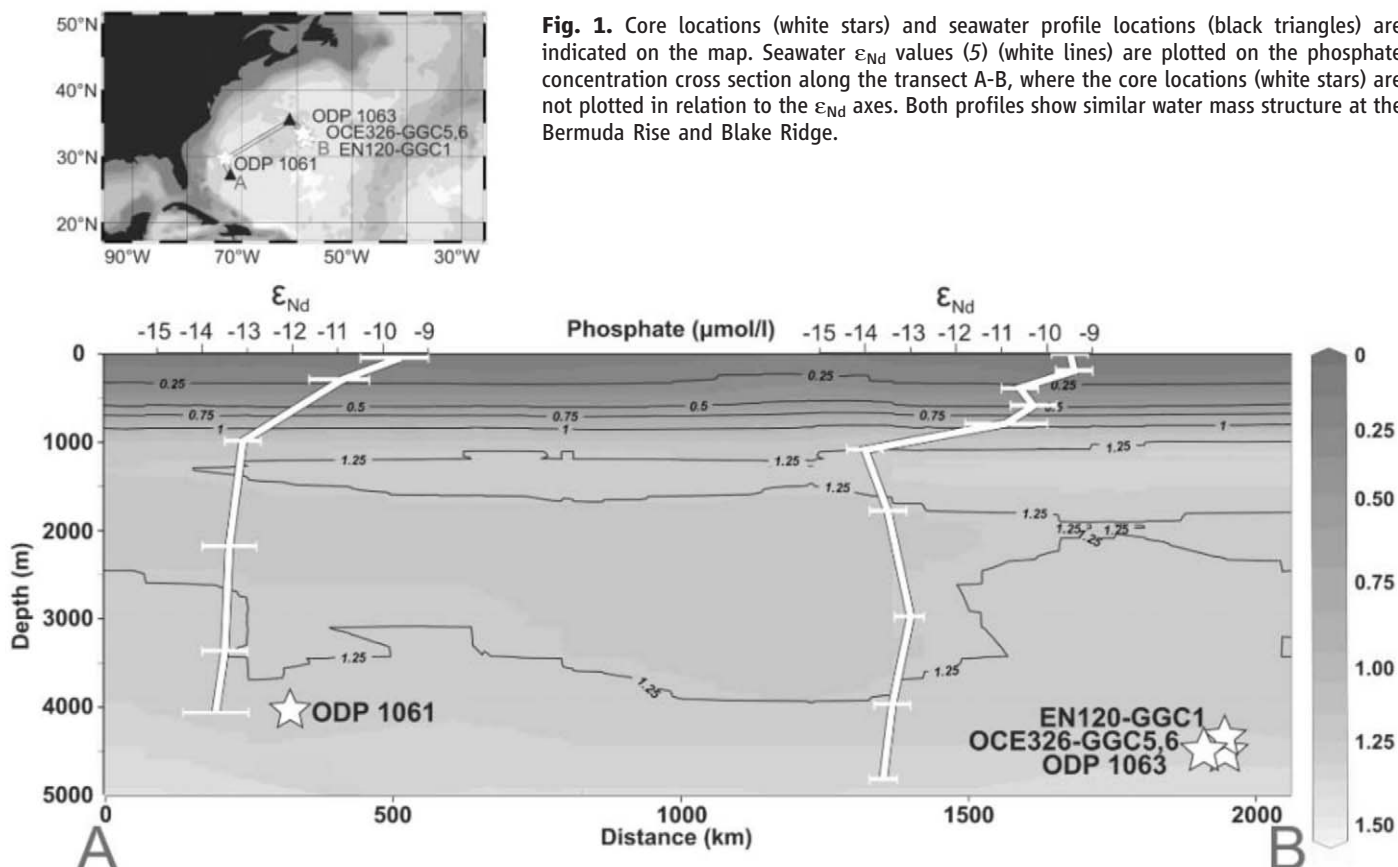


Fig. 1. Core locations (white stars) and seawater profile locations (black triangles) are indicated on the map. Seawater ϵ_{Nd} values (5) (white lines) are plotted on the phosphate concentration cross section along the transect A-B, where the core locations (white stars) are not plotted in relation to the ϵ_{Nd} axes. Both profiles show similar water mass structure at the Bermuda Rise and Blake Ridge.

isotopic signatures, complicating reconstruction of deep water composition. This is particularly problematic for sediment drift deposits. The Bermuda Rise has deposition rates reaching 100 cm per 1000 years, with $>63\text{ }\mu\text{m}$ -sized fraction as low as 0.25%, allowing submillennial resolution, but also indicating sediment transport (11, 12). Alternative phases such as fish teeth (13) or benthic foraminiferal calcite (14) are typically in low abundance, precluding high-resolution records. In this study we developed an earlier method established by Palmer and Elderfield (15) using Fe-Mn oxide coatings on planktonic foraminifera, which are underpinned by fish teeth measurements where possible, from core OCE326-GGC6 (GGC6; $33^{\circ}41.443'\text{N}$, $57^{\circ}34.559'\text{W}$, 4541 m) (9).

Previous studies have shown that Nd is incorporated into foraminiferal calcite at very low concentrations (16, 17); for example, off the coast of Somalia, plankton tow Nd concentrations average 0.11 ppm (17), relative to core top measurements in this study of 2.63 ppm. Thus, high Nd concentrations exhibited by planktonic foraminifera from sediment cores likely result from metal oxide coatings on the shell (17). The precipitation of Fe-Mn oxide coatings onto sediment particles has been demonstrated to take place at the sediment-water interface (10, 15). Dissolved Nd concentrations increase with depth in the water column (18), and because sediment particles are in contact with Nd-rich bottom waters far longer than with other water masses, they retain a bottom water signature. All reductively cleaned and uncleaned foraminifera [calcite plus coating, with all clay and silicates removed (9)] at three North Atlantic core tops are within error of each other and the bottom water (NADW) composition (Fig. 2). The reductively cleaned foraminifera fall along mixing curves predicted by plankton tow end members, in the direction of surface water concentrations and ϵ_{Nd} values. We calculate that in order to obtain surface water values, between 90 and $>98\%$ of all coatings must be removed; any remaining coatings are likely due to inefficient cleaning or readsorption of Nd onto clean calcite during cleaning (17). Because planktonic foraminifera Fe-Mn oxide coatings accurately archive bottom water ϵ_{Nd} at the Bermuda Rise, this may be a promising approach in regions of high sediment accumulation (9).

Downcore Nd isotope measurements were made on bulk sediment leachate (10), reductively cleaned and uncleaned planktonic foraminifera, and reductively cleaned fish debris from Bermuda Rise core GGC6 (Fig. 3). Although core top bulk sediment leachate ϵ_{Nd} values are within error of modern-day NADW (4, 5) (Fig. 2), downcore measurements are always more positive than the foraminiferal and fish debris ϵ_{Nd} and do not record the same changes as benthic $\delta^{13}\text{C}$ (*Cibicides wuellerstorfi*, epibenthic) from nearby Bermuda Rise core EN120-GGC1 ($33^{\circ}40'\text{N}$; $57^{\circ}37'\text{W}$, 4450 m) (19). This suggests variable addition of a radiogenic Nd contribution to the

Fig. 2. Core top measurements of reductively cleaned (open symbols) and uncleaned planktonic foraminifera (solid symbols) from cores ODP172 1061 (circles), OCE326-GGC6 (triangles), and ODP172 1063 (squares). Mixing curves are plotted on the basis of surface and deep water ϵ_{Nd} measurements (5) and average plankton tow Nd concentrations (0.11064 ppm; center dashed line) and minimum and maximum envelopes (0.01224 ppm and 0.34992 ppm, respectively) (17), and the maximum measured Fe-Mn coating Nd concentration. Surface water (light gray) and bottom water (dark gray) ϵ_{Nd} values are indicated by bands including 2σ errors (5). Numbers denote the fraction of Fe-Mn oxide coatings contributing to the ϵ_{Nd} signal.

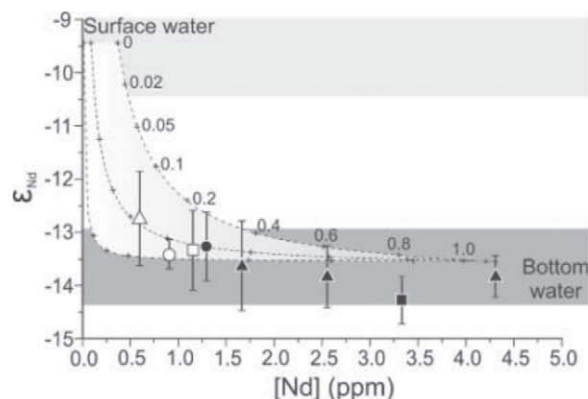
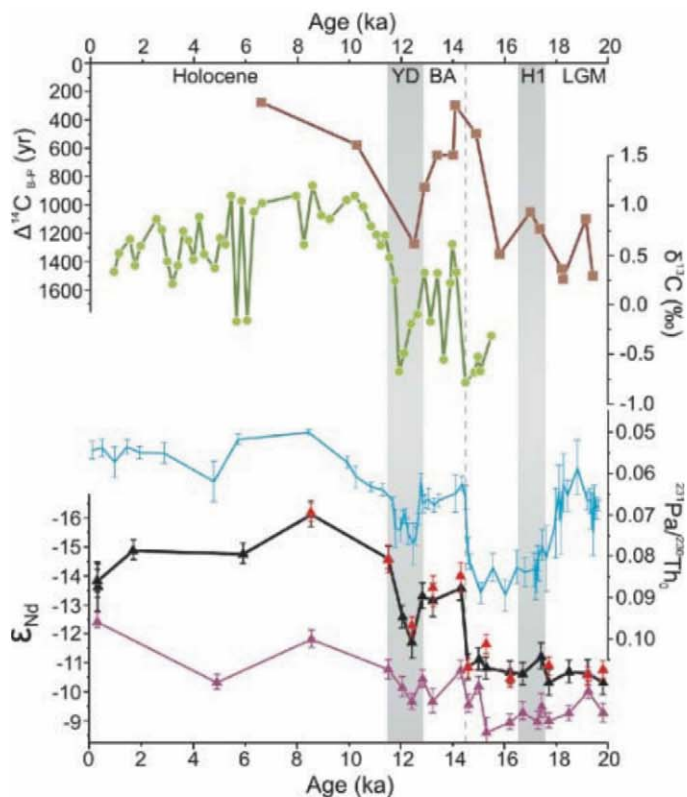


Fig. 3. GGC6 sediment leachates (purple triangles), uncleaned foraminifera (black triangles), and reductively cleaned fish debris (red triangles), plotted with GGC5 $^{231}\text{Pa}/^{230}\text{Th}_0$ ratios (blue line) (22), GGC1 benthic $\delta^{13}\text{C}$ (green circles) (19; for age model see (9)), and $\Delta^{14}\text{C}$ paired benthic and planktonic apparent ventilation ages (brown squares) (25, 26). The Holocene, Younger Dryas (YD), Bølling-Allerød (BA), Heinrich event 1 (H1), and 14,600 years ago (gray dashed line) are indicated; ka, thousands of years ago. Modern NADW $\epsilon_{\text{Nd}} \sim -14$ and AABW $\epsilon_{\text{Nd}} \sim -7$ to -9 (4, 5, 7).



bulk sediment leachate record. One plausible source is volcanic ash transported southward by bottom currents (11), providing a source of more positive Nd isotopic composition mobilized during leaching. Because foraminifera are not transported horizontally by currents (11), the observation that the uncleaned foraminifera and fish debris have no significant deviation from a 1:1 relationship throughout the record (fig. S2) confirms that we are measuring Fe-Mn oxide coatings precipitated on the planktonic foraminifera shells in bottom waters at the Bermuda Rise, as does the finding that core top samples match local deep water. The overall trend of the uncleaned foraminifera Nd isotopes ($\epsilon_{\text{Nd-UF}}$), from more radiogenic southern source deep water

(SSW) values in the glacial to less radiogenic northern source values in the Holocene, is consistent with benthic $\delta^{13}\text{C}$ at this site and elsewhere (20).

Changes in the strength of AMOC are believed to cause variations in the $^{231}\text{Pa}/^{230}\text{Th}_0$ ratio (21, 22) because faster overturning rates will result in a higher flux of Pa to the Southern Ocean relative to more particle-reactive Th, lowering the sedimentary $^{231}\text{Pa}/^{230}\text{Th}_0$ ratio in the North Atlantic (23). Measurements of planktonic and benthic $^{12}\text{C}/^{14}\text{C}$ ($\Delta^{14}\text{C}_{\text{B-P}}$) provide another means of estimating AMOC rate, because the age difference between the two samples indicates the time since ventilation. However, these proxies are not without ambiguities. Sedimentary $^{231}\text{Pa}/^{230}\text{Th}_0$

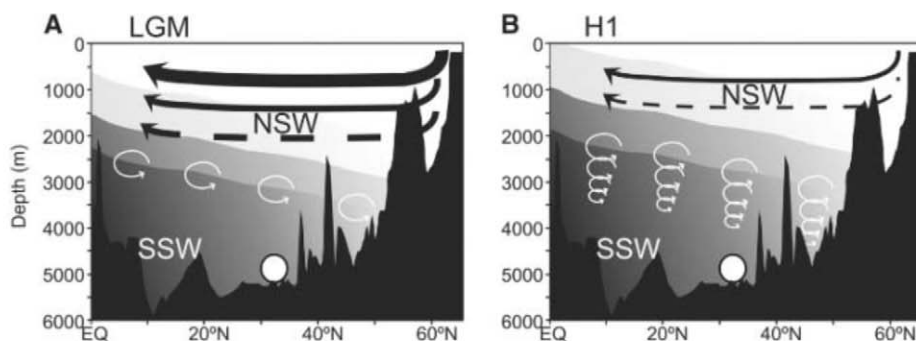


Fig. 4. Schematic illustration of ocean circulation over the Bermuda Rise (white circle) at (A) the LGM, and (B) Heinrich event 1. ϵ_{Nd} is represented by grayscale from more negative (light gray) to less negative (dark gray), with the relative overturning strength of NSW (black arrows) and the relative effect of vertical mixing on water mass ϵ_{Nd} (white arrows).

records may also be influenced by variable scavenging due to variations in particle flux and sediment composition, particularly opal (24). Deep water $\Delta^{14}C$ can also be influenced by the mixing of potentially varying and poorly constrained end members. The ϵ_{Nd-UF} and $^{231}Pa/^{230}Th_0$ shifts are sharp, coincident, and correlated across the deglacial climate transitions (the Bølling-Allerød–Younger Dryas–Holocene variations). ϵ_{Nd-UF} trends also match a composite $\Delta^{14}C_{B-P}$ apparent ventilation record produced from benthic and planktonic foraminifera and bivalves from deep sites throughout the western North Atlantic, confirming that these are basinwide changes (25, 26).

The ϵ_{Nd-UF} record (Fig. 3) allows us to make inferences about how AMOC source changes relate to ventilation and overturning rate. The Last Glacial Maximum (LGM) ϵ_{Nd-UF} data record a bottom water composition of ~ -10.6 , indicating the influence of SSW in the deep glacial Atlantic, an interpretation supported by $\delta^{13}C$ data (2, 20) and leachate Nd isotope data from the South Atlantic Cape Basin (10). Both Nd isotopes and $\Delta^{14}C_{B-P}$ remained constant from 20,000 to 14,600 years ago, indicating unchanged water mass source and overturning strength in the deep between the LGM and Heinrich event 1. Conversely, $^{231}Pa/^{230}Th_0$ shifted to higher values by 17,500 years ago, which was interpreted as a strong reduction in AMOC by McManus *et al.* (22). The fact that ϵ_{Nd-UF} did not reach SSW end-member values (27), combined with a constant gradient of 3.5 ϵ units between the Bermuda Rise and the Cape Basin (10), suggests that some proportion of northern source water (NSW) was continuously advected southward and is evidence against a total shutdown of AMOC. It also suggests that $^{231}Pa/^{230}Th_0$ ratios that approach the production ratio must at least in part be due to opal fluxes increasing the ratio (28), but does not fully elucidate the effect of opal flux. However, with evidence of moderate opal fluxes at the LGM (28), any correction on $^{231}Pa/^{230}Th_0$ necessitates LGM values that indicate overturning similar to or stronger than the Holocene or Bølling-Allerød. Because the deep $\Delta^{14}C_{B-P}$ and

ϵ_{Nd-UF} records see a relatively small contribution from NSW during the LGM, $^{231}Pa/^{230}Th_0$ is likely integrating the entire water column and primarily recording fast, shallow overturning of glacial intermediate NSW (22). This is concordant with increasing evidence from other proxies supporting a fast, shallow overturning cell at the LGM (20, 23, 26, 29, 30).

Our results support the necessity for relatively fast overturning at the LGM in order to maintain a steep vertical chemical gradient in the north-west Atlantic (20) under conditions of strong vertical mixing (31) (Fig. 4A). If freshwater perturbation induced by Heinrich event 1 slowed overturning, it affected only the upper ocean (30), resulting in constant deep $\Delta^{14}C_{B-P}$ ventilation rates and an increase in the $^{231}Pa/^{230}Th_0$ ratio integrated through the water column. With slower overturning, the strong vertical mixing would have weakened the chemical gradient, allowing more NSW to be transported to depth. This would have offset any effect of decreased NSW production and shoaling of AABW, and would have resulted in no significant change in the Nd isotopic signature at this site (Fig. 4B). Because there were no changes in the boundary conditions at this time, Heinrich event 1 may be likened to modeling results indicating relatively gradual changes in overturning rates during glacial freshwater perturbation (32).

In contrast, all three deep water proxies, as well as the integrated $^{231}Pa/^{230}Th_0$ ratio shift during the deglacial events starting at 14,600 years ago, invoke whole-ocean change in both overturning rate and water mass source, similar to jumps in state via hysteresis behavior (32). This is evidence for two different ocean circulation responses to climate forcing, which suggests that glacial millennial-scale changes in shallow overturning are inherently different from Milankovitch-forced glacial-interglacial changes, because the latter are accompanied by shifts in boundary conditions and whole water column reorganization.

The large deglacial shifts in benthic $\delta^{13}C$ and the constant ϵ_{Nd} gradient between the North and South Atlantic from 20,000 to 14,600 years ago suggest no change in the NSW end member.

However, the early Holocene ϵ_{Nd} value of -16 recorded by foraminifera and fish teeth indicates that NSW was 2 ϵ units more negative than modern NADW. This suggests an increased proportion of the Labrador Sea component of NADW, which is thought to have begun advecting at ~ 8000 years ago (33).

Fe-Mn oxide coatings that are precipitated onto planktonic foraminifera reliably record bottom water ϵ_{Nd} at the Bermuda Rise today, and provide an accurate archive of past water mass structure changes in areas of sediment redistribution. If meltwater at Heinrich event 1 did affect ocean overturning, it was most likely in the upper part of the water column, enhancing the effects of vertical mixing and resulting in unchanging chemical signatures in the deep. This calls for modeling studies that distinguish between intermediate and deep overturning changes and do not invoke total shutdown of AMOC. After 14,600 years ago we observe coupled North Atlantic water mass source and overturning strength shifts through deglacial climate events linking the relative proportion of NSW and SSW with rates of overturning. There is invariably a strong ocean-climate link over the last deglacial, but the sensitivity of this link must be tested during more stable times (for example, the Holocene) in order to determine future climate change implications.

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Human Genome Sequencing Using Unchained Base Reads on Self-Assembling DNA Nanoarrays

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Genome sequencing of large numbers of individuals promises to advance the understanding, treatment, and prevention of human diseases, among other applications. We describe a genome sequencing platform that achieves efficient imaging and low reagent consumption with combinatorial probe anchor ligation chemistry to independently assay each base from patterned nanoarrays of self-assembling DNA nanoballs. We sequenced three human genomes with this platform, generating an average of 45- to 87-fold coverage per genome and identifying 3.2 to 4.5 million sequence variants per genome. Validation of one genome data set demonstrates a sequence accuracy of about 1 false variant per 100 kilobases. The high accuracy, affordable cost of \$4400 for sequencing consumables, and scalability of this platform enable complete human genome sequencing for the detection of rare variants in large-scale genetic studies.

Genotyping technologies have enabled the routine assessment of common genetic variants at up to a million sites across the genome in thousands of individuals (1) and have increased our understanding of human genetic diversity and its biological and medical impact. Whole-genome sequencing costs have

dropped from the >\$100 million cost of the first human genomes (2, 3) to the point where individual labs have generated genome sequences in a matter of months for material costs of as low as \$48,000 (4–12) (table S5). Sequencing technologies, which use a variety of genomic microarray construction methodologies and sequencing chemistries (13–32), can determine human genetic diversity over an entire genome and identify common as well as rare single-nucleotide polymorphisms (SNPs), insertions, and deletions. Despite these advances, improvements are still needed to enable the cost-effective characterization of the many hundreds of genomes required for genetic studies of complex diseases and for personalized disease prevention, prognosis, and treatment.

We generated sequencing substrates [Fig. 1A and supporting online material (SOM)] by means of genomic DNA (gDNA) fragmentation and recursive cutting with type IIS restriction

enzymes and directional adapter insertion (Fig. 1B and fig. S1). The resulting circles were then replicated with *Phi*29 polymerase (RCR) (33). Using a controlled, synchronized synthesis, we obtained hundreds of tandem copies of the sequencing substrate in palindrome-promoted coils of single-stranded DNA, referred to as DNA nanoballs (DNBs) (Fig. 1C). DNBs were adsorbed onto photolithographically etched, surface-modified (SOM) 25- by 75-mm silicon substrates with grid-patterned arrays of ~300-nm spots for DNB binding (Fig. 1C). The use of patterned arrays increased DNA content per array and image information density relative to random genomic DNA arrays (6, 9, 11, 14, 28). High-accuracy combinatorial probe anchor ligation (cPAL) sequencing chemistry was then used to independently read up to 10 bases adjacent to each of eight anchor sites (Fig. 1D), resulting in a total of 31- to 35-base mate-paired reads (62 to 70 bases per DNB). cPAL is based on unchained hybridization and ligation technology (15, 27, 28, 31), previously used to read 6 to 7 bases from each of four adapter sites (26 total bases) (28), here extended using degenerate anchors to read up to 10 bases adjacent to each of the eight inserted adapter sites (Fig. 1D, right) with similar accuracy at all read positions (fig. S3). This increased read length is essential for human genome sequencing.

Cell lines derived from two individuals previously characterized by the HapMap Project (34), a Caucasian male of European descent (NA07022) and a Yoruban female (NA19240), were sequenced. NA19240 was selected to allow for a comparison of our sequence to the sequence of the same genome currently being assembled by the 1000 Genome Project. In addition, lymphoblast DNA from a Personal Genome Project Caucasian male sample, PGP1 (NA20431) was sequenced because substantial data are available for biological comparisons (35–37). Automated cluster analysis of the four-dimensional intensity data produced raw base reads and associated raw base scores (SOM).

We mapped these sequence reads to the human genome reference assembly with a custom alignment algorithm that accommodates our read structure (fig. S4), resulting in between 124 and 241 Gb mapped and an overall genome coverage of 45- to 87-fold per genome.

To assess representational biases during circle construction, we assayed genomic DNA and intermediate steps in the library construction process by quantitative polymerase chain reaction

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(QPCR) (fig. S2). This and mapped coverage showed a substantial deviation from Poisson expectation with excesses of both high and low coverage regions (fig. S5), but only a few percent of bases have coverage insufficient for assembly (Table 1). Much of this coverage bias is accounted for by local GC content in NA07022, a bias that was significantly reduced by improved adapter ligation and PCR conditions in NA19240 (fig. S5); the fraction of the genome with less

than 15-fold coverage was accordingly reduced from 11% in NA07022 to 6.4% in NA19240, despite the latter having 25% less total coverage (Table 1).
Discordance with respect to the reference genome in uniquely mapping reads from NA07022 was 2.1% (range 1.4% to 3.3% per slide). However, considering only the highest scoring 85% of base calls reduced the raw read discordance to 0.47%, including about 0.1% of true variant positions.

Mapped reads were assembled into a best-fit, diploid sequence with a custom software suite employing both Bayesian and de Bruijn graph techniques (SOM). This process yielded diploid reference, variant, or no-calls at each genomic location with associated variant quality scores. Confident diploid calls were made for 86% to 95% of the reference genome (Table 1), approaching the 98% that can be reconstructed in simulations. The 2% that is not reconstructed

Fig. 1. Amplified DNA nanoarray platform. (A) Schematic flow diagram of the process used. (B) Library construction schematic (fig. S1). r1 to r8 are gDNA regions adjacent to distinct adapter ends; Ad1 to Ad4 indicate adapters 1 to 4. (C) DNB production using *Phi*29 DNA polymerase (fig. S11) and nanoarray formation (SOM) schematics. (D) Schematic of cPAL products (SOM).

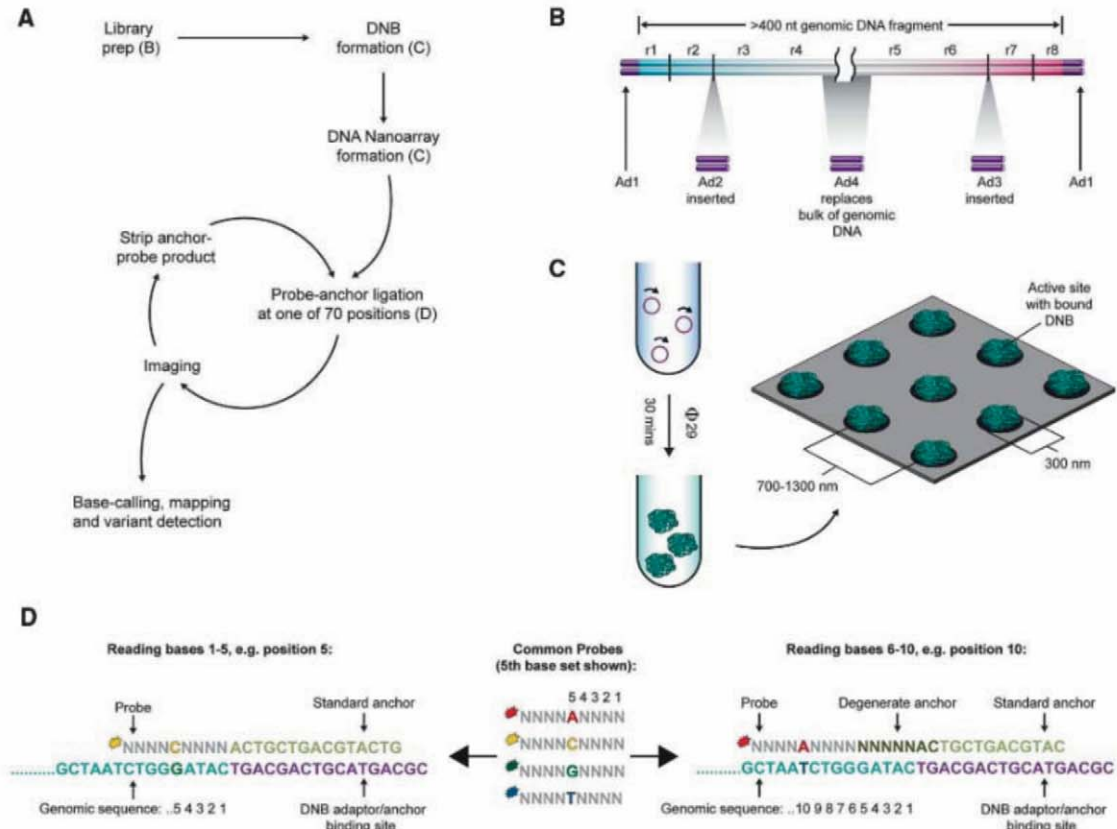


Table 1. Summary information from mapping and assembly of three genomes. All variations are with respect to the National Center for Biotechnology Information (NCBI) version 36 human genome reference assembly. Novel variations were ascertained by comparison to dbSNP [JDW, release 126; NA18507 (6), release 128; all other genomes, re-

lease 129]. NA18507 and NA19240 are Yoruban HapMap samples, which may explain the number of SNPs and novelty rates. In partially called regions of the genome, one allele could be called confidently but not the other. The high call rate in NA19240 reflects reduced library bias (fig. S5).

Sample	Mapped sequence (Gb)	Average coverage depth (fold)	Percent of genome called		SNPs		Indels		Insertion: deletion ratio
			Fully	Partially	Total	Novel	Total	Novel	
Genomes sequenced by Complete Genomics									
NA07022 (35)	241	87	91%	2%	3,076,869	10%	337,635	37%	1.0
NA19240 (36)	178	63	95%	1%	4,042,801	19%	496,194	42%	0.96
NA20431 (37)	124	45	86%	3%	2,905,517	10%	269,794	37%	1.0
Genomes previously published									
NA18507 (6)	131	46	—	—	4,139,196	26%	404,416	50%	0.77
NA18507 (9)	87	31*	—	—	3,866,085	19%	226,529	33%	0.72
JCV (3)	21	7	—	—	3,213,401	15%	851,575	—	—
JDW (4)	21	7	—	—	3,322,093	18%	222,718	51%	0.4

*This is 18x when constrained to nonduplicated and properly mated reads, which were those used for variant calling.

Table 2. Concordance with genotypes for NA07022 generated by the HapMap Project (release 24) and the highest quality Infinium assay subset of those genotypes, as well as genotyping on Illumina Infinium 1M assay. Discordances with reported HapMap Infinium genotypes were verified by Sanger sequencing (SOM).

		Infinium 1M	HapMap phase I and II SNPs	HapMap Infinium subset	HapMap Infinium SNPs tested for accuracy by Sanger sequencing			
Published concordance*		—	99.03%	99.94%				
NA07022	Number reported		1 M	3.9 M	143 K			
	Called (%)		95.98%	94.39%	96.00%	These data correct	These data incorrect	Affirmed (%)
	Locus concordance (%)		99.89%	99.15%	99.88%			
	HapMap	Homozygous reference	99.96%	99.34%	99.96%	18	2	90%
	genotype	Heterozygous	99.78%	99.39%	99.80%	28	46	38%†
	calls	Homozygous alternate	99.81%	98.14%	99.84%	28	12	70%†

*Published concordance between Sanger sequencing and genotyping (34). †Average false negative rate is <0.19.

in simulations is composed of repeats that are longer than the ~400 base inserts used here and of high enough identity to prevent attribution of mappings to specific repeat copies. Longer mate-pair inserts minimize this limitation (6, 9). Similar limitations affect other short-read technologies.

We identified a range of 2.91 to 4.04 million SNPs with respect to the reference genome, 81% to 90% of which are reported in the database dbSNP, as well as short indels and block substitutions (Table 1 and table S6). Because of the use of local de novo assembly, indels were detected in sizes ranging up to 50 bp. As expected, indels in coding regions tend to occur in multiples of length 3, indicating the possible selection of minimally impacting variants in coding regions (fig. S6).

As an initial test of sequence accuracy, we compared our called SNPs with the HapMap phase I/II SNP genotypes reported for NA07022 (1). We fully called 94% of these positions with an overall concordance of 99.15% (Table 2) (the remaining 6% of positions were either half-called or not called). Furthermore, we fully called 96% of the Infinium (Illumina, San Diego, CA) subset of the HapMap SNPs with an overall concordance rate of 99.88%, reflecting the higher reported accuracy of these genotypes (34). Similar concordance rates with available SNP genotypes were observed in NA19240 (with a call rate of over 98%) and NA20431 (table S7).

We further characterized 134 of the 168 calls that were discordant with Infinium loci and Sanger sequencing of PCR products in NA07022, demonstrating that 55% of these discordances are errors in the reported HapMap genotypes (Table 2). The relationship between detection rate and read depth for about 1M Infinium HD SNPs that we subsequently genotyped in NA07022 shows that coverage of 30-fold at a position is sufficient to detect 90% of SNPs at heterozygous loci and 97% of SNPs at homozygous loci (fig. S5).

Because the whole-genome false-positive rate cannot be accurately estimated from known SNP loci, we tested a random subset of novel nonsynonymous variants in NA07022, a category that is enriched for errors (10). We extrapolated error rates from the targeted sequencing of 291

Table 3. False-positive rates and false discovery rates (FDRs) were calculated for the entire set of variations called in NA07022 by extrapolating the heterozygous (Het) FDRs calculated from comparative Sanger sequencing of 291 selected novel variants (table S8) to all variants. This is a conservative approach (detailed in SOM). The total number of all types of false-positive variants is estimated at 7.5 to 16.1 per Mb.

Variation type	Total detected	Novel	Het novel FDR (table S8)	Estimated false positives on genome	Estimated false positives / Mbp	Estimated FDR
SNP	3,076,869	310,690	2–6%	7k–17k	2.3–6.1	0.2–0.6%
Deletion	168,726	61,960	8–14%	5k–8k	1.8–3.0	3.0–5.0%
Insertion	168,909	61,933	11–18%	7k–11k	2.3–3.9	3.9–6.5%
Block substitution	62,783	30,445	11–29%	3k–9k	1.1–3.1	5.2–13.9%

such loci and estimated the false-positive rate at about one variant per 100 kb, including <6.1 substitution-, <3.0 short deletion-, <3.9 short insertion- and <3.1 block variants per Mb (Table 3 and table S8).

Aberrant mate-pair gaps may indicate the presence of length-altering structural variants and rearrangements with respect to the reference genome. A total of 2,126 clusters of such anomalous mate-pairs were identified in NA07022. We performed PCR-based confirmation of one such heterozygous 1500-base deletion (fig. S7). More than half of the clusters are consistent in size with the addition or deletion of a single *Alu* repeat element.

Some applications of complete genome sequencing may benefit from maximal discovery rates, even at the cost of additional false positives, whereas for others, a lower discovery rate and lower false-positive rate may be preferable. We used the variant quality score to tune call rate and accuracy (fig. S8). Additionally, novelty rate (relative to the dbSNP) is also a function of variant quality score (fig. S9).

We processed the NA07022 data with Trait-o-matic (Scalable Computer Experts, Somerville, Massachusetts, and Church Laboratory, Harvard Medical School, Cambridge, Massachusetts) automated annotation software [as in (12)], yielding 1159 annotated variants, 14 of which may have disease implications (table S10).

Because the DNB sequencing substrates are produced by rolling-circle replication (33) in a uniform-temperature, solution-phase reaction with high template concentrations (>20 billion

per ml), this system avoids substantial selection bottlenecks and nonclonal DNBs. This circumvents the stochastic inefficiencies of approaches that require precise titration of template concentrations for in situ clonal amplification in emulsion (9, 14, 29) or bridge PCR (6, 19).

Our patterned arrays include high-occupancy and high-density nanoarrays self-assembled on photolithography-patterned, solid-phase substrates through electrostatic adsorption of solution-phase DNBs and yield a high proportion of informative pixels (site occupancies >95%) (fig. S12A) compared with random-position DNA arrays. This results in several hundred reaction sites in the compact (~300-nm diameter) DNB that produce bright signals useful for rapid imaging of the sequences (SOM). Such small DNBs also allow for high-density arrays. The data set reported herein was generated with arrays with ~350 million spots at a pitch of 1.29 μm. Such a spot density and higher ones achieved in proof-of-concept experiments (fig. S12B) result in high image efficiency and reduced reagent consumption that enable high sequencing throughput per instrument critical for high-scale human genome sequencing for research and clinical applications.

Both sequencing by synthesis (SBS) and sequencing by ligation (SBL) use chained reads, wherein the substrate for cycle *N* + 1 is dependent on the product of cycle *N*; consequently, errors may accumulate over multiple cycles and data quality may be affected by errors (especially incomplete extensions) occurring in previous cycles. Thus, reactions need to be driven to

near completion with high concentrations of expensive high-purity labeled substrate molecules and enzymes. The independent, unchained nature of cPAL avoids error accumulation and tolerates low-quality bases in otherwise high-quality reads, thereby decreasing reagent costs. The average sequencing consumables cost for these three genomes was under \$4400 (table S5). The raw base and variant call accuracy achieved compares favorably with other reported human genome sequences (2–12).

Because the sequencing substrates are produced by a DNA engineering process based on modified nick-translation for directional adapter insertion (SOM), we obtained over 90% yield in adapter ligation and low chimeric rates of about 1% (SOM). DNA molecules with an inserted adapter are further enriched with PCR (SOM). This recursive process can be implemented in batches of 96 samples and extended by inserting additional adapters to read 120 bases or more per DNB (fig. S10). The current read length is comparable to other massively parallel sequencing technologies (6–12).

The sequence data reported here achieve sufficient quality and accuracy for complete genome association studies, the identification of potentially rare variants associated with disease or therapeutic treatments, and the identification of somatic mutations. The low cost of consumables and efficient imaging may enable studies of hundreds of individuals. The higher

accuracy and completeness required for clinical diagnostic applications provides an incentive for continued improvement of this and other technologies.

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Supporting Online Material

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Structure and Mechanisms of a Protein-Based Organelle in *Escherichia coli*

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Many bacterial cells contain proteinaceous microcompartments that act as simple organelles by sequestering specific metabolic processes involving volatile or toxic metabolites. Here we report the three-dimensional (3D) crystal structures, with resolutions between 1.65 and 2.5 angstroms, of the four homologous proteins (EutS, EutL, EutK, and EutM) that are thought to be the major shell constituents of a functionally complex ethanolamine utilization (Eut) microcompartment. The Eut microcompartment is used to sequester the metabolism of ethanolamine in bacteria such as *Escherichia coli* and *Salmonella enterica*. The four Eut shell proteins share an overall similar 3D fold, but they have distinguishing structural features that help explain the specific roles they play in the microcompartment. For example, EutL undergoes a conformational change that is probably involved in gating molecular transport through shell protein pores, whereas structural evidence suggests that EutK might bind a nucleic acid component. Together these structures give mechanistic insight into bacterial microcompartments.

Bacterial microcompartments are present in diverse bacteria, where they function as protein-based organelles (1–6). They range in size from just under 1000 Å to around 1500 Å, and they typically have a polyhedral shape (Fig. 1A). Each type of microcompartment contains a few different enzymes that catalyze sequential metabolic reactions. The enzymes are encapsulated by a shell formed from a few thousand shell protein

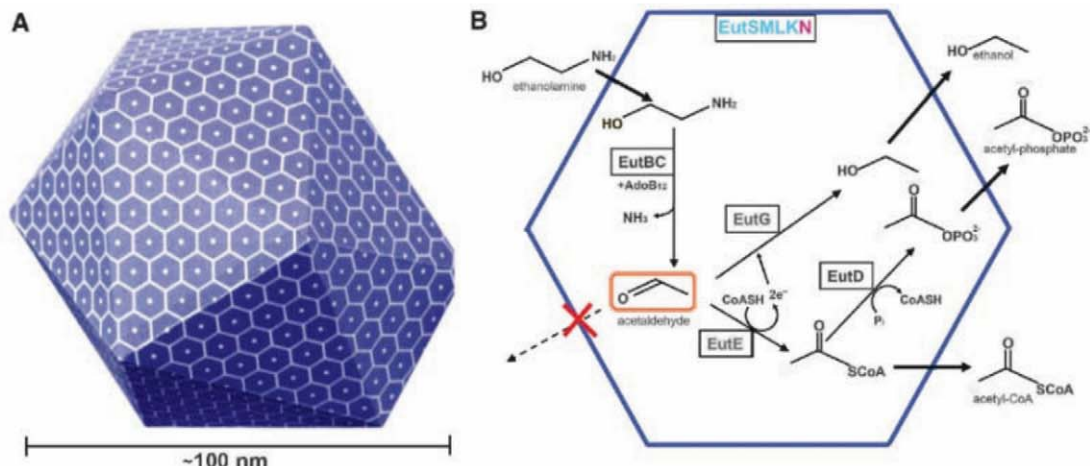
subunits. The simplest microcompartment is the carboxysome, which encapsulates the two enzymes carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) in order to enhance cellular CO₂ fixation (1, 7, 8). Recent structural studies on the carboxysome and its shell proteins have provided a basic understanding of how that type of microcompartment is assembled and how it operates (9–14).

Other bacterial microcompartments with more complex metabolic functions have also been discovered (5, 15). One of these, which is dedicated to ethanolamine utilization (Eut), is present in several bacteria, including *Salmonella enterica* and *Escherichia coli* (4, 16). The Eut microcompartment shares a number of homologous enzymes with a propanediol utilization (Pdu) microcompartment; both metabolic pathways proceed via aldehyde intermediates, propionaldehyde in the case of Pdu and acetaldehyde in the case of Eut (3). Experiments in *Salmonella* have shown that the cellular function of the Eut microcompartment is to metabolize ethanolamine without allowing the release of acetaldehyde into the cytosol (Fig. 1B), thus mitigating the potentially toxic effects of excess aldehyde in the bacterial cytosol (17–19) and also preventing the volatile acetaldehyde from diffusing across the cell membrane and leading to a loss of carbon (20).

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Fig. 1. A model for the Eut microcompartment and its metabolic pathway. **(A)** A hypothetical model of the Eut microcompartment emphasizing the construction of a semiregular polyhedron primarily from hexameric shell proteins. **(B)** A model for the metabolism of ethanolamine in the Eut microcompartment. Ethanolamine is converted into ethanol, acetyl-phosphate, and acetyl-coenzyme A (CoA), to be used in the tricarboxylic acid cycle for energy production. The volatile intermediate, acetaldehyde (boxed in orange), is consumed before it can escape the protein shell. The four homologous shell proteins belonging to the conserved BMC family (EutK, EutL, EutM, and EutS) are colored in light blue. The unrelated EutN protein (dark pink) may be a minor structural component of the shell, based on analogy to the carboxysome (11). Enzymes thought to be associated with the



microcompartment are indicated. Protein names: EutBC, ethanolamine ammonia-lyase; EutD, phosphotransacetylase; EutE, aldehyde dehydrogenase; EutG, alcohol dehydrogenase. The *eut* operon (fig. S1) encodes other enzymes and proteins involved in ethanolamine utilization, which are not shown.

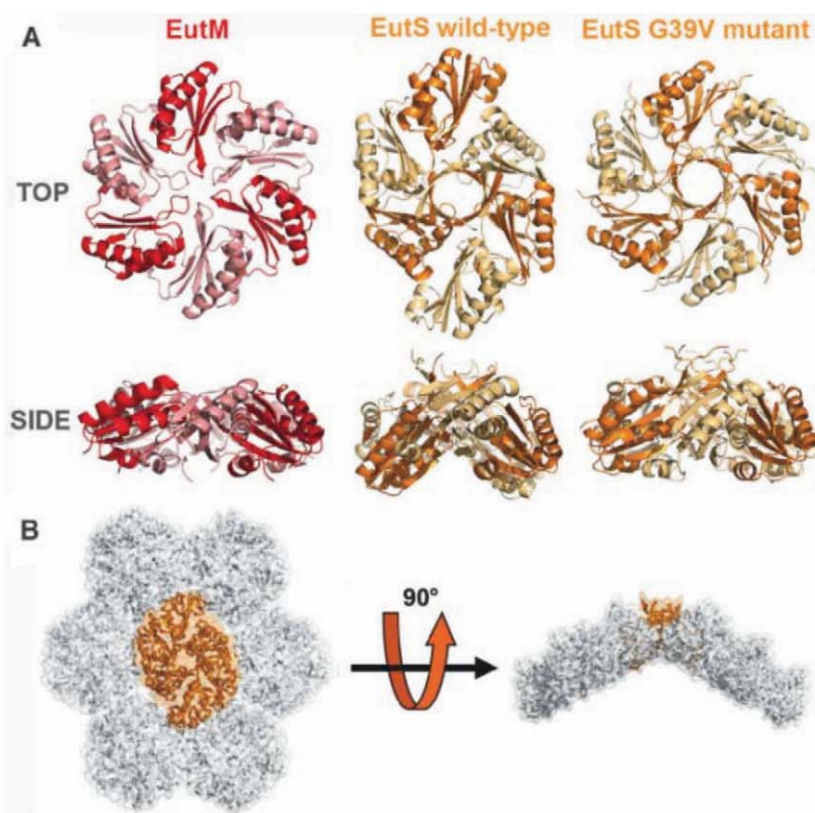


Fig. 2. Structure of EutM and EutS shell protein hexamers. **(A)** Cartoon diagrams of the EutM (left), EutS (middle), and mutant EutS G39V (right) hexamers, shown in two views. The wild-type EutS hexamer is bent away from a flat configuration by approximately 40° (SOM text). This asymmetric configuration of wild-type EutS was converted into a flat symmetric configuration by the G39V mutation. **(B)** A hypothetical model showing how EutS (orange) might introduce curvature in an otherwise flat hexameric sheet of shell protein hexamers. Hexamer interfaces in the model are based on packing arrangements that are conserved in other crystal structures. From a row of EutS hexamers at an edge, flat hexamers could extend to form facets of the shell without major collisions.

The major shell proteins from known bacterial microcompartments belong to a family of homologous proteins that are typically just over 100 amino

acids long, referred to here as bacterial microcompartment (BMC) proteins. The amino-acid sequences of BMC proteins were determined first

from microcompartments of the carboxysome type (21), and can now be identified in some 40 genera across the bacterial kingdom (2, 3). In *Salmonella* and *E. coli*, the 17-gene *eut* operon codes for four homologous BMC proteins: EutK, EutL, EutM, and EutS [(4) and supporting online material (SOM) text]. Genetic studies in *Salmonella* have shown that EutL, EutM, and EutK are required for growth on ethanolamine when it is the sole carbon source at high pH, and their deletion leads to rapid loss of acetaldehyde (20). Likewise, deleting EutS led to a measurable loss of acetaldehyde but did not cause a growth phenotype under the conditions examined. A fifth protein, EutN, which is probably a minor shell component but not homologous to the BMC shell proteins, was also shown to be essential for growth on ethanolamine; its structure was reported previously (11). Here we report the crystal structures of the BMC shell proteins from *E. coli*: EutM, EutS (wild-type and mutant), EutL (in two crystal forms), and a domain of EutK. The Eut shell proteins from *E. coli* share between 79 and 96% sequence identity with their orthologs from *Salmonella* (fig. S1 and SOM text), where functional studies have been performed (17, 18, 20).

The crystal structure of EutM was determined to a resolution of 2.1 Å. The 97-amino acid EutM protein adopts a 3D fold with an α/β structure that matches closely those reported earlier for proteins forming the shell of the carboxysome (9, 10). Six EutM subunits self-assemble to make a flat cyclic hexamer with a bowl-shaped depression on one side, punctuated by a narrow central pore (Fig. 2A). Similar hexameric structures have been established as the basic building blocks from which microcompartment shells are assembled by the tight packing of many hexamers side by side into a molecular layer or sheet (Fig. 1A) (9, 10, 12). Electron density, interpreted to be a sulfate ion from the crystallization mixture, was visualized in the center of the EutM pore, in accordance with previous suggestions that small molecules and ions can

pass through the centers of the hexameric shell proteins (fig. S2) (9, 10). The relative abundances of the various shell proteins in the Eut microcompartment have not been established, but the EutM protein hexamer, with its relatively typical features, could serve as the basic assembly component of the shell, whereas the other shell proteins, whose unusual features we describe below, could perform more-specialized roles.

The crystal structure of EutS was determined at 1.65 Å resolution. The 111-amino acid protein adopts the conserved α/β core structure expected for BMC proteins (Fig. 2A), but the secondary structure elements occur in nonsequential order relative to the typical BMC fold. Similar circular permutations of the BMC protein fold have been observed previously (22–24). Unlike other BMC proteins that have formed flat hexameric structures, the EutS hexamer has a bend of approximately 40° (Fig. 2 and SOM text). As a result, the six chemically

equivalent protein subunits exist in three distinct structural environments. The closest parallels to this asymmetric assembly are to the proteins of certain viral capsids in which equivalent protein subunits are present in quasiequivalent, but distinct, environments (25, 26). The observation could be helpful in explaining microcompartment architecture, because previously observed (flat) BMC hexamers have not clarified how the edges of polyhedral microcompartment shells might be formed.

The cause of the bent EutS structure was investigated by mutagenesis. A glycine residue (Gly³⁹) involved in a crystal contact was mutated to a valine side chain (G39V). Valine is the corresponding amino acid in a homologous shell protein from the Pdu microcompartment, namely PduU, which forms a flat symmetric hexamer (22). The G39V mutant of EutS migrated more slowly in a native gel (despite an unaltered net charge), implicating a substantial conformational change (fig.

S3). That observation was consistent with a crystal structure that demonstrated that the mutated EutS was flattened into a symmetric structure (Fig. 2A). Thus Gly³⁹ appears to play an important role in bending EutS. Gly³⁹ is conserved among EutS proteins from the *eut* operon of other bacteria but is absent from other BMC proteins.

EutL is the longest of the Eut shell proteins. A recent structure (24) showed that it contains within a single polypeptide chain two tandem domains (Fig. 3A), with each domain being a circular permutation of the typical BMC fold (22), similar to EutS. As such, three copies of EutL assemble to form a pseudohexameric structure. We determined the crystal structure of EutL from two distinct crystal forms, both at a resolution of 2.3 Å. A comparison of the two structures revealed that protein backbone movements of nearly 15 Å (in loops covering residues 65 to 83 and 174 to 185) interconvert the EutL trimer between forms in which the central pore is either open or closed (Fig. 3B). The conformation for EutL identified here as being the closed form matches the one described earlier (24). The central pore is very nearly occluded. Very small openings in this structure were noted earlier as potential routes for transport, but it is likely the open form observed here that is competent for transport. The pore in the open form is triangular in shape, with an edge of approximately 11 Å and a diameter at its narrowest point of about 8 Å. This large pore is salient, because it provides a potential route for transporting bulky molecules such as cobalamin and other nucleotide cofactors that are required by reactions inside the microcompartment (3) (Fig. 1B). Model building suggests that the open pore could accommodate transport of these cofactors with only modest side-chain movements (fig. S6). The existence of a closed form is also potentially important, because the possibility of a triggered or gated opening goes partway toward addressing the paradox of how relatively large molecules might penetrate the shell without allowing rapid loss of the small acetaldehyde intermediate (3). A potential mechanism for

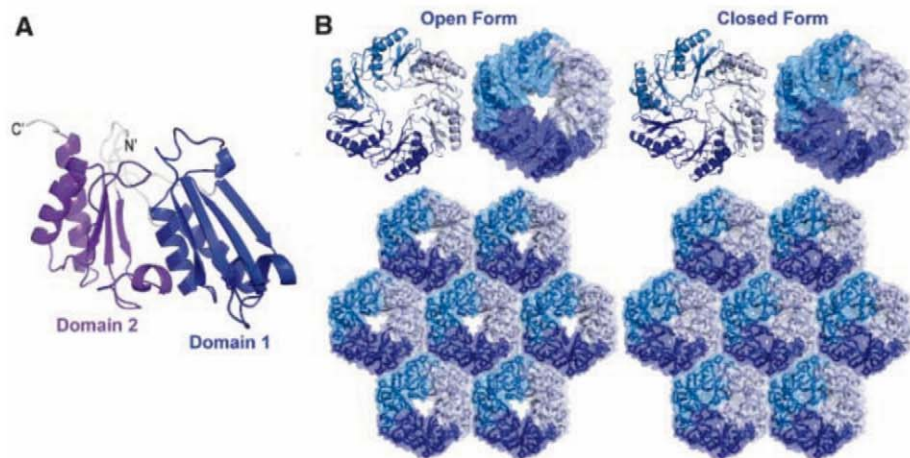
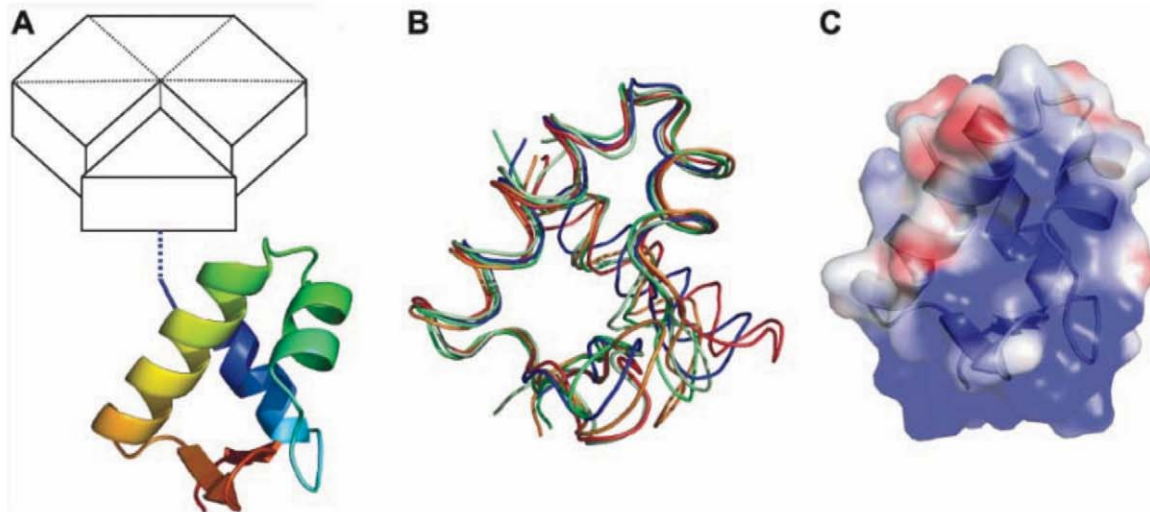


Fig. 3. The structure of the EutL shell protein and its gated pore. (A) A cartoon ribbon diagram of a EutL monomer in its closed form. The first and second BMC domains are colored in blue and purple, respectively. (B) The open (left) and closed (right) configurations of EutL trimers are shown in both ribbon diagram and surface representations. In both configurations, EutL trimers (or pseudohexamers) were observed to pack into tight molecular layers within their respective crystal forms (bottom). The conformational change between forms involves 15 Å movements of the protein backbone (fig. S4).

Fig. 4. The structure of the EutK Ctail. (A) A schematic diagram of the BMC domain and the crystal structure of the C-terminal helix-turn-helix domain of EutK, interpreted as a monomer (fig. S7). (B) Superimpositions of the structure of the EutK-Ctail on four representative DNA/RNA binding domains (SOM text, colored according to fig. S9). (C) An electrostatically colored surface diagram of the EutK-Ctail domain emphasizing the positively charged (blue) patch that is characteristic of DNA binding domains.



selective transport would involve a coupling between cofactor binding and pore opening. A similar conformational change was reported recently in a carboxysome shell protein, CsoS1D (23) (figs. S4 and S5), suggesting that gated transport could be a common mechanism in microcompartments.

Like some other BMC proteins, EutL was found to associate into tightly packed molecular layers within both of the crystal forms characterized here (Fig. 3B). This observation lends further support to models for microcompartment architecture in which the shell is formed by the tight side-by-side packing of hexameric (or pseudohexameric) protein building blocks into a molecular layer or sheet (9–11). The appearance of the EutL protein layer, composed of subunits in either the open or closed configuration, illustrates how strongly the porosity of the microcompartment would be affected by the conformational change in EutL (Fig. 3B).

The fourth shell protein, EutK, is distinct among the shell proteins in the Eut microcompartment. First, although all other BMC proteins studied to date form hexamers (or pseudohexamers, like EutL), EutK is a monomer in solution (fig. S8). The apparent inability of EutK to assemble into a hexamer by itself suggests that different BMC paralogs might form mixed hexamers during assembly of the shell. Second, EutK has an extra protein domain of about 60 amino acids following the conserved BMC-type domain (Fig. 4A). Numerous instances of BMC-type proteins fused to other uncharacterized protein domains are evident in the protein sequence databases, but structural and functional data are limited (27). Based on sequence similarity of marginal statistical certainty (for example, a 40% probability of similarity due to random chance), the extra domain of EutK could be only tentatively assigned to a broad family of proteins bearing a well-known helix-turn-helix motif, which is common among nucleic acid-binding proteins.

Whereas crystals could not be grown using the full-length EutK protein, the crystal structure of the C-terminal domain was elucidated at a resolution of 2.1 Å. The crystal structure of this 59–amino acid fragment, referred to hereafter as EutK-Ctail, demonstrates that it is indeed a helix-turn-helix domain (Fig. 4B). A computational search for similar structures in the protein structure database identified many known helix-turn-helix domains as close matches, with coordinate differences as low as 1.0 Å. About 90% of the helix-turn-helix domain structures retrieved from the search bind nucleic acids, whereas a minority perform other varied cellular functions (SOM text). A comparison of the surface features of EutK-Ctail with previously characterized helix-turn-helix domains, including those that bind nucleic acids and those that do not, suggests strongly that EutK is a nucleic acid-binding protein. A prominent, positively charged surface is conserved in EutK-Ctail and in those domains that bind nucleic acids (Fig. 4C). Interestingly, on the basis of structure-guided alignments, EutK-Ctail shows the highest se-

quence similarity to the helix-turn-helix domains of the IclR family of transcription factors, which regulate, among other things, carbon metabolism in enterobacteria (28, 29) (fig. S9). The specific function of EutK and the identity of its cognate nucleic acid are unknown. Nonetheless, the key prediction that the Eut microcompartment shell binds a nucleic acid points up the possibility of unexpected parallels to viral capsids, which bind to and encapsulate their viral genomes.

Taken together, the structures of the shell proteins from the Eut microcompartment paint a picture of a complex, highly evolved organelle. The structural features and conformational changes observed illustrate how these evolutionarily related proteins have diverged to fulfill specialized architectural and biochemical roles in a shell that participates actively in the function of the microcompartment.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S9

Tables S1 to S3

References

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The Tasmanian Devil Transcriptome Reveals Schwann Cell Origins of a Clonally Transmissible Cancer

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The Tasmanian devil, a marsupial carnivore, is endangered because of the emergence of a transmissible cancer known as devil facial tumor disease (DFTD). This fatal cancer is clonally derived and is an allograft transmitted between devils by biting. We performed a large-scale genetic analysis of DFTD with microsatellite genotyping, a mitochondrial genome analysis, and deep sequencing of the DFTD transcriptome and microRNAs. These studies confirm that DFTD is a monophyletic clonally transmissible tumor and suggest that the disease is of Schwann cell origin. On the basis of these results, we have generated a diagnostic marker for DFTD and identify a suite of genes relevant to DFTD pathology and transmission. We provide a genomic data set for the Tasmanian devil that is applicable to cancer diagnosis, disease evolution, and conservation biology.

Devil facial tumor disease (DFTD) is a transmissible cancer affecting the Tasmanian devil (*Sarcophilus harrisii*), an endemic Tasmanian marsupial carnivore. First

observed in 1996 in northeastern Tasmania, DFTD has been implicated in devil population collapse (1, 2). DFTD is a rapidly fatal disease that culminates in large tumors, primarily on the face

and mouth, which frequently metastasize to internal organs (3). There are no diagnostic tests, treatments, or vaccines available for DFTD, and models predict that the disease could lead to extinction of wild Tasmanian devils within 25 to 35 years (4).

DFTD appears to be a clonal cell line, transmitted (by biting) as an allograft between devils (5, 6) and may be similar in transmission to canine transmissible venereal tumor (CTVT) and a transmissible sarcoma affecting Syrian hamsters (7–9). The prevalence and biology of such somatic cell parasites is generally unknown (10).

Studies of captive Tasmanian devils have suggested that the species is prone to developing tumors, particularly carcinomas (11, 12). However, DFTD does not resemble previously described devil cancers (3, 13), and determining its etiology is critical for developing management strategies for the disease. Cytologically, DFTD appears as a soft tissue neoplasm consisting of undifferentiated round to spindle-shaped cells with few defining ultrastructural features (3, 13). Immunohistochemistry suggests that the tumor is derived from neuroectoderm (13).

Clonal transmission of DFTD was proposed on the basis of karyotypic evidence (5) and was supported by genetic analysis of DFTD tumors at microsatellite and major histocompatibility complex loci (6). We genotyped at 14 microsatellite loci 25 paired tumor and host samples, as well as 10 samples from DFTD-unaffected devils from 16 locations in Tasmania (14) (figs. S1 and S2 and table S1). All DFTD tumors shared a similar genotype across all loci, regardless of location, sex, or age of the animal ($P = 0.18$, permutation test) (figs. S1 and S2). Furthermore, the tumor genotype was distinct from that of both the hosts and unaffected devils ($P < 0.001$, permutation test) (figs. S1 and S2). These data were consistent with previous studies (5, 6) and support the supposition that DFTD is a single clonal cell line propagated as a tumor allograft.

To further assess the clonal origin of DFTD, we sequenced a 1180–base pair fragment of the mitochondrial locus control region (LCR) from 14 tumors, 14 hosts, and 9 DFTD-unaffected devils (table S2). We found that all devils and tumors shared a single LCR haplotype in this region, except for one single-nucleotide polymorphism at position 15,711, which supported the idea that the tumors are clonal. Furthermore, this nucleotide variant was observed only in DFTD-free devils from western Tasmania (figs. S1 and S2 and table S2), a genetically distinct population (15). The karyotypic and genetic consistency between DFTD tumors (figs. S1 and S2) (5, 6) reinforces epidemiological evidence for a recent origin of DFTD (1, 3).

We cloned and deeply sequenced microRNA (miRNA) from 10 devil tissues and five DFTD tumors, including one DFTD mammary metastasis, to gain insight into the histogenesis of DFTD. The 114 Tasmanian devil miRNAs, identified with strict conservation parameters, showed characteristic tissue-specific expression profiles (Fig. 1 and table S3). DFTD had a relatively consistent miRNA profile that was distinct from the other 10 tissues (Fig. 1 and fig.

S3). Differential expression of four miRNAs in DFTD relative to a non-DFTD tissue (testis) was confirmed by quantitative polymerase chain reaction (PCR) (fig. S4). Hierarchical clustering based on Pearson's correlation statistic showed that the DFTD tumors were clustered (Fig. 1 and table S4) and that the non-DFTD miRNA profile most highly correlated with DFTD was that of brain (Fig. 1 and table S4).

In cancer, miRNAs can both promote and suppress tumors, as well as regulate processes including cell proliferation, angiogenesis, and metastasis (16). It is noteworthy that the DFTD profile included a number of miRNAs commonly up-regulated in tumors, including *miR-21*, *miR-24*, and *miR-19b* (16), plus a miRNA that has been linked to tumor immune evasion (*miR-222*) (17) (Fig. 1 and fig. S3). In contrast, DFTD expresses very low levels of *miR-29b* and *miR-126*, two miRNAs suggested to suppress tumors (Fig. 1 and fig. S3) (16).

To create a catalog of genes expressed in DFTD, we sequenced the transcriptome of DFTD and testis (chosen because of its expression of a broad range of genes) from an individual Tasmanian devil resulting in 13,665 and 16,438

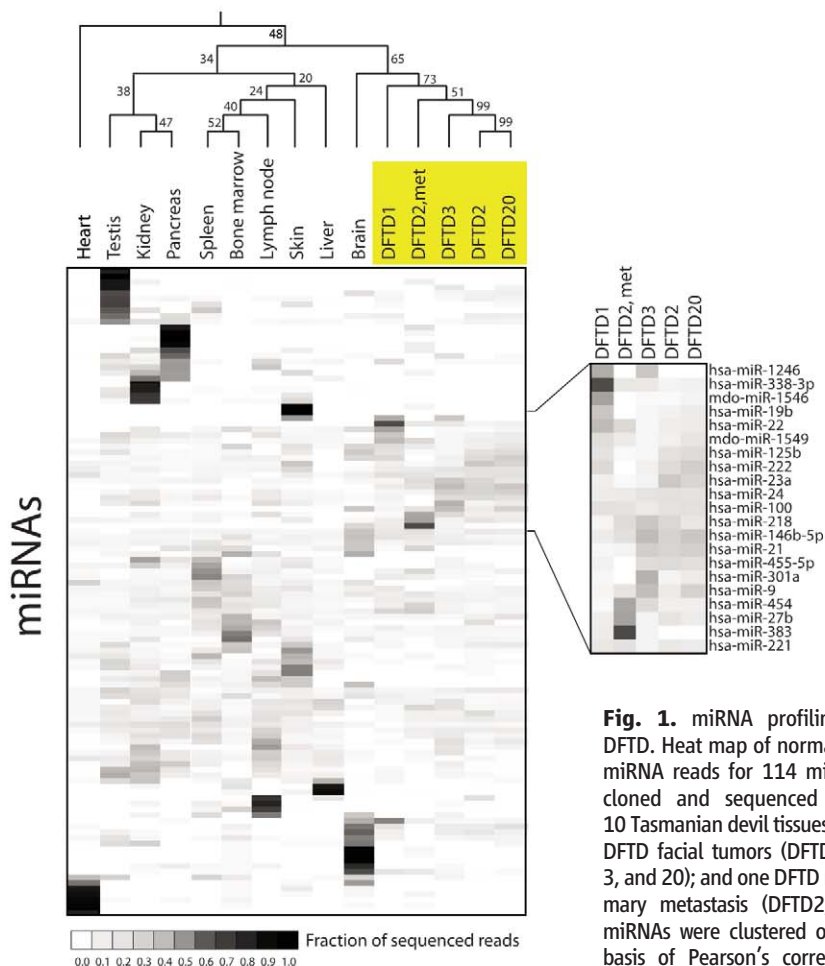


Fig. 1. miRNA profiling of DFTD. Heat map of normalized miRNA reads for 114 miRNAs cloned and sequenced from 10 Tasmanian devil tissues; four DFTD facial tumors (DFTD1, 2, 3, and 20); and one DFTD mammary metastasis (DFTD2,met). miRNAs were clustered on the basis of Pearson's correlation

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statistic, and bootstrap values (percentage) are indicated. The DFTD miRNA profile is shown in greater detail. miRNAs were annotated on the basis of conservation with comparison species hsa, human; mdo, opossum.

unique transcripts identified on the basis of similarity with human proteins and opossum transcripts, respectively (see SOM text). Of the annotated transcripts, 0.4% were differentially represented between tumor and testis libraries (fig. S5). The 31 transcripts with the most significant enrichment in the tumor library (tumor-to-testis ratio of at least 2.5, chi-squared test $P \leq 0.05$) were validated by semiquantitative PCR (Fig. 2A). Of these, 20 transcripts were at least twice as highly expressed in tumor as in testis (Fig. 2A).

The gene with the highest expression in DFTD relative to a housekeeping gene, *GAPDH* (encoding glyceraldehyde-3-phosphate dehydrogenase), was *MBP*, the gene that encodes myelin basic protein (Fig. 2A). Indeed, 9 of the 20 validated tumor genes (45%) were found to be involved in the myelination pathway (table S6). Myelin, an insulating membranous layer that ensheathes nerve axons, is produced by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system. Components of a molecular network that controls the differentiation of Schwann cells, including transcription factors (*SOX10*, *SOX2*, *POU5F1*, and *JUN*) and structural myelin genes (*MPZ*, *PRX*, *MBP*, and *PMP22*), were apparent in the differentially expressed DFTD transcriptome (Fig. 2A) (18).

We also measured the expression of 31 tumor-enriched genes from a panel of 10 Tasmanian devil tissues by semiquantitative PCR. Hierarchical clustering of this data set grouped DFTD together with peripheral nerves, a tissue enriched for Schwann cells (Fig. 2B). To confirm the expression of myelin proteins in DFTD, we stained DFTD tumor tissues with the antibody against Schwann cell-specific myelin protein, periaxin (*PRX*) (Fig. 3). All tumor cells in DFTD lesions, as well as the myelinated sheaths of peripheral nerve bundles, were strongly and specifically positive for *PRX* (Fig. 3). We also detected protein expression in DFTD of myelin proteins *MBP*, *MPZ*, and *PMP22*, as well as *NES*, *NGFR*, and *S100* (figs. S6 and S7, and table S7). These experiments confirm that DFTD expresses genes found in myelinating cells, including Schwann cell-specific markers (*PRX*, *PMP22*, and *MPZ*).

At present, diagnosis of DFTD is based on clinical features and histology. This can pose particular difficulties for the diagnosis of atypical DFTD such as nonfacial DFTD or DFTD metastases. We tested *PRX* as a potential diagnostic marker for DFTD by staining a panel of both DFTD and non-DFTD Tasmanian devil tumors with *PRX*. All ($n = 20$) of the DFTD tumors were positive for *PRX*, whereas none of the nine non-DFTD tumors tested were positive (table S7). In addition, all ($n = 10$) of the DFTD metastases collected from a variety of organs were positive for *PRX* (table S7). Thus, *PRX* is a strong and specific marker for DFTD and is suitable for diagnostic evaluation.

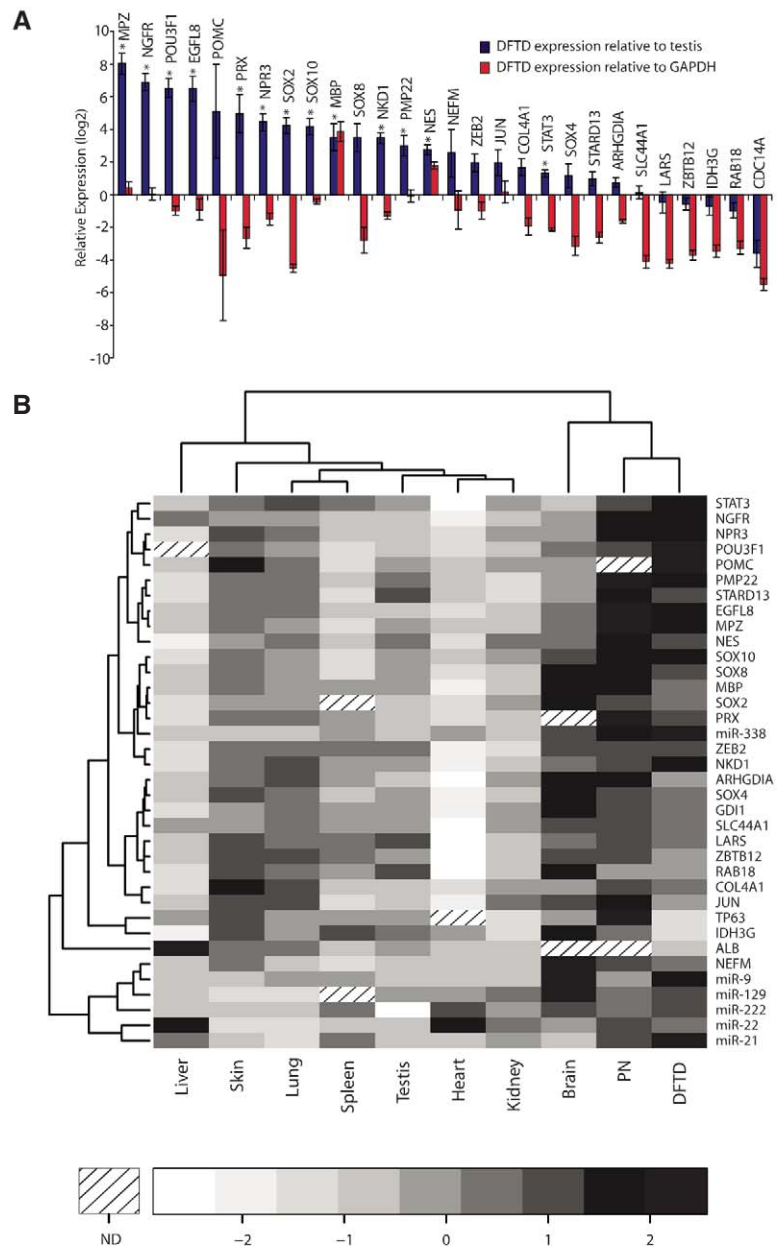


Fig. 2. DFTD transcriptome. **(A)** Semiquantitative RT-PCR expression profiling of 31 genes with enriched expression in tumor relative to testis [454-read count fold change ≥ 2.5 , $P \leq 0.05$, chi-squared test; (green points in fig. S5)]. Log values of the mean expression difference of DFTD genes relative to testis (blue bars) and relative to *GAPDH* (red bars) are shown. Error bars represent standard deviation. Significant differences between tumor and testis expression levels ($P \leq 0.05$) are indicated by an asterisk (two-sample *t* test, Holm's correction for multiple testing). **(B)** Heat map of semiquantitative PCR expression profiles of 31 genes across a panel of tissues including peripheral nerve (PN), a Schwann cell-enriched tissue. Panel color represents the mean gene expression level, standardized across tissues (*z* score). Hierarchical clustering based on Pearson's correlation statistic is indicated by dendrograms. For each tissue three biological replicates were performed ($n = 3$), not determined.

It is striking that DFTD, a cytologically undifferentiated tumor, expresses markers of highly differentiated Schwann cells because human Schwann cell tumors rarely express a complete set of terminally differentiated myelin genes (19). Although it is possible that Schwann cell genes have been activated in DFTD cells after carcinogenic transformation,

it is more likely that the myelin program observed here reflects the DFTD cell of origin. We therefore propose that DFTD is a peripheral nerve sheath tumor that arose from a Schwann cell or Schwann cell precursor; this is supported by the miRNA profile of DFTD (Figs. 1 and 2). Schwann cells participate in nerve repair after injury and also modulate

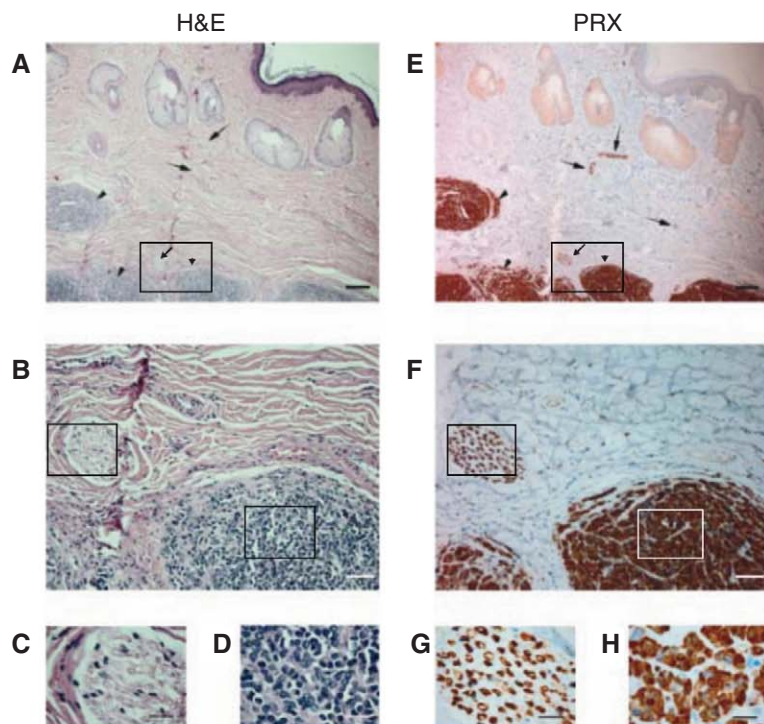


Fig. 3. Identification of a diagnostic marker for DFTD. (A to D) Hematoxylin and eosin (H&E) and (E to H) PRX antibody stains used for DFTD tumor histology. Arrowheads, DFTD tissue; arrows, peripheral nerve bundles (containing Schwann cells). Magnification, 4× (A and E; scale bar, 200 μm); 20× (B and F; scale bar, 50 μm); and 100× (C and G) peripheral nerve bundle (scale bar, 20 μm) and (D and H) DFTD tumor (scale bar, 20 μm). Boxed areas indicate approximate locations of areas magnified in lower panels.

local immune reactions in the peripheral nervous system (20, 21). The plasticity and immuno-competence of Schwann cells may be significant in the evolution of DFTD as a transmissible cancer.

Interestingly, *proopiomelanocortin* (*POMC*), a gene encoding a peptide hormone precursor, was expressed in DFTD (Fig. 2). This transcript varies by more than 100-fold between tumors (fig. S8). Technical limitations, due to lack of antibody cross-reactivity with devil, prevented the detection of physiological levels of adrenocorticotrophic hormone (ACTH), a labile *POMC* cleavage product. However, compared with healthy individuals, diseased devils had higher, but not significantly different, levels of serum cortisol (fig. S9, $P = 0.06$, Student's t test). Cortisol is a steroid hormone, released by ACTH activity, that is involved in stress responses and has immunosuppressive activity (22). These studies raise the intriguing possibility that DFTD may secrete factors that could alter host physiology and/or behavior, an interesting area for further study.

Our study highlights the activities of two miRNA gene networks that may function in DFTD. *PMP22*, a myelin gene expressed in DFTD, is a target of *miR-29*, a miRNA that is down-regulated in DFTD (Figs. 1 and 2 and fig. S3) (23). The expression of transcription factor *ZEB2* is negatively correlated with low

expression of the *miR-200* family, which controls tumor invasiveness in a negative-feedback loop (24) (Figs. 1 and 2 and fig. S3). Furthermore, this work suggests that commonly mutated Schwann cell cancer genes, such as *NF1*, are interesting candidates for further analysis in DFTD (25).

DFTD is believed to be of neuroendocrine origin on the basis of the expression of the vimentin, S100, neuron-specific enolase, chromogranin A, and synaptophysin markers (13). As Schwann cells and neuroendocrine cells are both derived from the neural crest and overlap in gene expression, it is possible that DFTD has elements of both tissue types.

A Schwann cell origin for DFTD contrasts with that of the canine clonally transmissible cancer, which has been proposed to be of histiocytic origin (26, 27). It will be of interest to define common and unique features of these two cancers and to determine how the histogenesis of transmissible cancers may influence their occurrence, evolution, and biology. Our catalog of genes provides a framework for this work, as well as for use in the effort to develop a DFTD preclinical test and vaccine.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5961/84/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 to S11
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O-Mannosyl Phosphorylation of Alpha-Dystroglycan Is Required for Laminin Binding

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Alpha-dystroglycan (α -DG) is a cell-surface glycoprotein that acts as a receptor for both extracellular matrix proteins containing laminin-G domains and certain arenaviruses. Receptor binding is thought to be mediated by a posttranslational modification, and defective binding with laminin underlies a subclass of congenital muscular dystrophy. Using mass spectrometry- and nuclear magnetic resonance (NMR)-based structural analyses, we identified a phosphorylated O-mannosyl glycan on the mucin-like domain of recombinant α -DG, which was required for laminin binding. We demonstrated that patients with muscle-eye-brain disease and Fukuyama congenital muscular dystrophy, as well as mice with myodystrophy, commonly have defects in a postphosphorylation modification of this phosphorylated O-linked mannose, and that this modification is mediated by the like-acetylglucosaminyltransferase (LARGE) protein. These findings expand our understanding of the mechanisms that underlie congenital muscular dystrophy.

Diverse posttranslational modifications influence the structure and function of many proteins. Dystroglycan (DG) is a membrane protein that requires extensive posttranslational processing in order to function as an extracellular matrix receptor. It is composed of an extracellular α -DG subunit and a transmembrane β -DG subunit (1). α -DG serves as a receptor for extracellular matrix laminin G domain-containing ligands such as laminin (1) and agrin (2) in both muscle and brain, and these interactions depend on an unidentified posttranslational α -DG modification. α -DG is also the cellular receptor for lymphocytic choriomeningitis virus (LCMV), Lassa fever virus (LFV), and clade C New World arenaviruses (3, 4). Although the binding sites for LCMV

and LFV on α -DG have not yet been identified, they are thought to overlap with the modification recognized by laminin (5, 6).

Glycosyltransferase-mediated glycosylation is one type of posttranslational modification with two main forms in mammals: N- and O-glycosylation, which are distinguished by how the oligosaccharide moiety links to the amino acid. Mutations in six known or putative glycosyltransferase genes—*protein O-mannosyl transferase 1 (POMT1)* (7), *POMT2* (8), *protein O-mannose beta-1,2-N-acetylglucosaminyltransferase 1 (POMGnT1)* (9), *fukutin* (10), *fukutin-related protein (FKRP)* (11), and *LARGE* (12)—have been identified in patients with congenital muscular dystrophy (CMD). These disorders affect the brain, eye, and skeletal muscle to different extents, the most severe being Walker-Warburg syndrome [WWS; Online Mendelian Inheritance in Man (OMIM) identification number (ID) 236670], with less severe phenotypes seen in muscle-eye-brain disease (MEB; OMIM ID 253280) and Fukuyama CMD (FCMD; OMIM ID 253800). In these diseases, the ability of α -DG to bind laminin is markedly reduced (13), suggesting that these (putative) glycosyltransferases participate in the posttranslational modification that enables α -DG to bind laminin. Whereas the molecular functions of LARGE, fukutin, and FKRP remain unclear, POMT1 and -2 (14) and POMGnT1 (9) are known to catalyze two steps in the biosynthesis of an O-mannosyl tetrasaccharide (NeuNAc- α -2,3-Gal- β -1,4-GlcNAc- β -1,2-Man) that is found in high abundance on both brain and muscle α -DG (15, 16). However, this glycan itself is probably not the laminin-binding moiety, because glycosidase-mediated removal of the glycan does not reduce α -DG binding to laminin (17).

To determine which posttranslational modification is necessary for the α -DG/laminin interaction, we processed wheat germ agglutinin-

enriched proteins (glycoproteins) from C57BL/6J (wild-type, WT) muscle using various enzymatic and chemical treatments. Treatment with cold aqueous hydrofluoric acid (HFAq), which specifically cleaves phosphoester linkages (18), resulted in the reduction of the α -DG relative molecular mass (M_r) from 150 to 70 kD, the loss of IIH6 immunoreactivity and laminin binding (Fig. 1A), and the loss of binding to LFV and LCMV (Fig. 1B). Because the M_r of N-glycosylated β -DG did not change (Fig. 1A), these effects were not caused by the degradation of either peptide or glycosyl linkages. A quantitative solid-phase assay revealed a 97% reduction in total high-affinity binding to laminin (Fig. 1C). HFAq treatment also abolished the laminin-receptor activity of α -DG in the heart, brain, and kidney (fig. S1A). We next tested whether N-glycan and/or the two O-glycans known to modify the laminin-binding form of α -DG—Core1 O-glycan and the O-mannosyl tetrasaccharide (in either the sialylated or fucosylated form) (15, 16)—are sensitive to HFAq treatment. Immunoblotting of WT muscle glycoproteins treated with several cocktails of glycosidases that degrade these three glycans showed that the glycosidase-mediated reduction in α -DG glycosylation was impervious to HFAq treatment (Fig. 1D). A similar experiment using muscle glycoproteins from the CMD-model mouse *LARGE^{myd}*, in which a mutation in LARGE prevents the α -DG modification necessary for laminin-binding (19), revealed that HFAq treatment did not significantly reduce the M_r of α -DG (Fig. 1D). Thus, HFAq specifically degrades the laminin-binding moiety on α -DG. Further, functional modification of α -DG appeared to involve an internal phosphoryl linkage rather than a monoester-linked phosphate, because digesting WT muscle glycoproteins with alkaline phosphatase did not reduce the laminin-binding ability (fig. S1B).

To verify that α -DG is phosphorylated, we labeled human embryonic kidney (HEK293) cells expressing Fc-tagged α -DG recombinants (Fig. 2A) that are secreted into the medium with [³²P]-orthophosphate. Phosphorimaging showed that secreted DGFc4, which contains only the mucin-like region of α -DG (20), was phosphorylated (Fig. 2B). Hydrolysis of [³²P]-DGFc4 under conditions that are conducive to the dissolution of polypeptide and phosphoester linkages to carbohydrates, but not to linkages to amino acids (21), generated inorganic phosphate but not phospho-amino acids (fig. S2), suggesting that phosphorylation does not occur directly on the peptide. To test whether the phosphorylation depends on glycosylation, we expressed DGFc5 in [³²P]-orthophosphate-labeled human cells derived from *POMT1*-mutated WWS, *POMGnT1*-mutated MEB, *fukutin*-mutated FCMD, and control cells, as well as in fibroblasts from *LARGE^{myd}* and WT mice (Fig. 2C). All except the *POMT1*-mutated WWS cells secreted [³²P]-phosphorylated DGFc5 into the medium, strongly suggesting that phosphorylation occurs on the O-linked mannose

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Fig. 1. Chemical dephosphorylation by HFaQ treatment abolishes laminin and virus binding to α -DG in tissues from WT mice. (**A** and **B**) Treated glycoproteins prepared from WT muscle were subjected to the following: (**A**) immunoblotting with antibodies against the α -DG core protein (CORE) or the laminin-binding form α -DG epitope (IIH6) and β -DG, or to laminin overlay assay; (**B**) virus overlay assays with γ -inactivated LFV or LCMV cl-13. (**C**) WT muscle glycoproteins with and without HFaQ treatment were subjected to a solid-phase laminin-binding assay ($n = 3$). Open circles, treated; solid circles, untreated. Error bars indicate SD. (**D**) Muscle glycoproteins from WT and *Large^{myd}* (*Myd*) mice were digested with cocktails of glycosidases that degrade sialylated and/or fucosylated *N*-glycan, Core 1 *O*-glycan, and *O*-mannosyl glycan, before (first four lanes) and after (last four lanes) HFaQ treatment. The products were subjected to either immunoblotting with CORE antibody or laminin overlay assay.

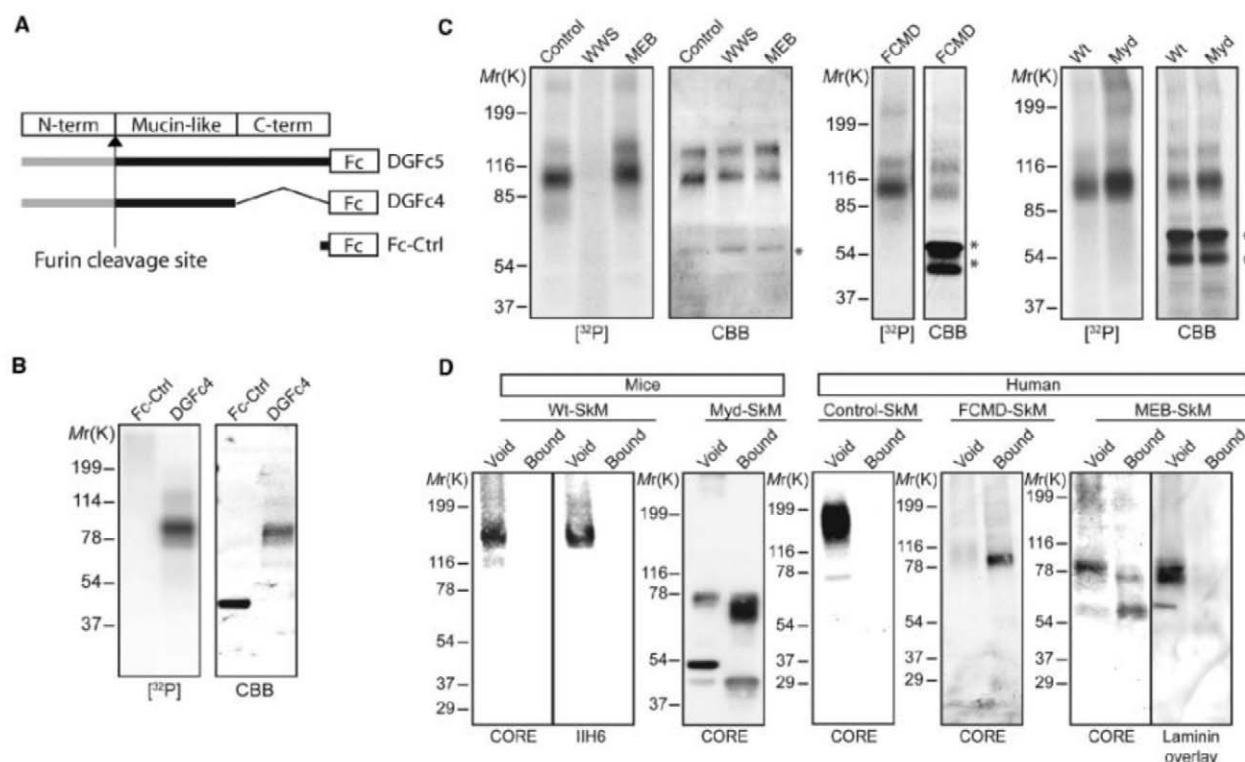
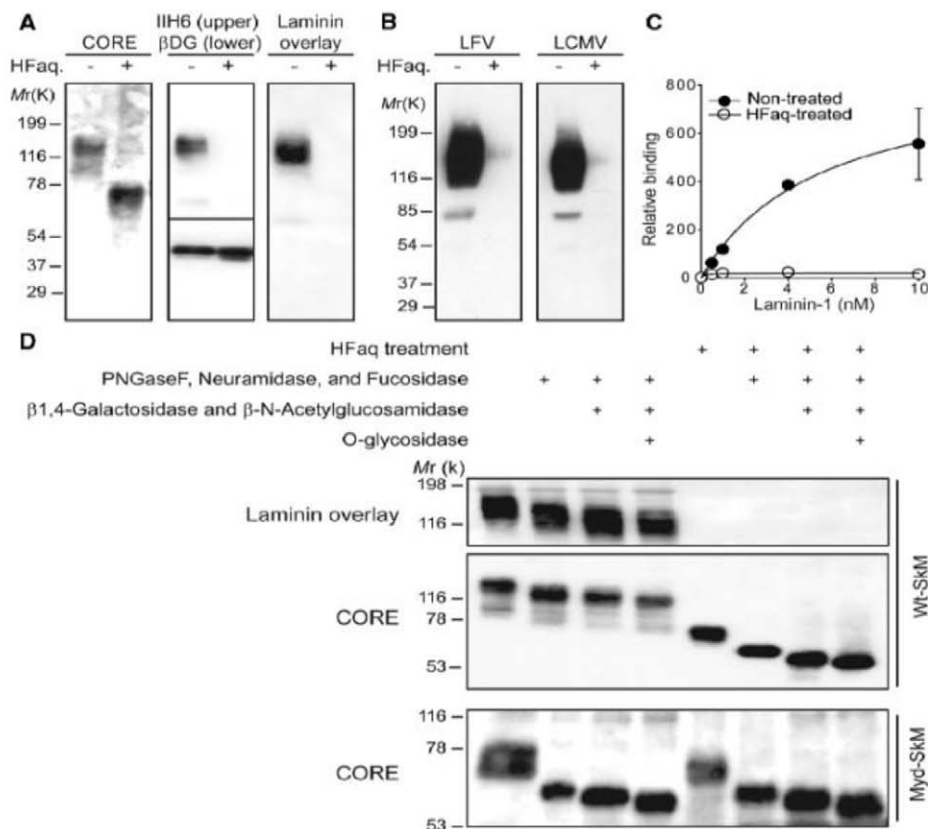


Fig. 2. The mucin-like domain of α -DG is phosphorylated in an *O*-mannosylation-dependent manner. (**A**) Structures of recombinant α -DG constructs used in the study. (**B** and **C**) 32 P-orthophosphate labeling of (B) Fc-Ctrl- or DGFc4-expressing HEK293 cells and (C) DGFc5-expressing cultured cells from CMD patients (WWS, MEB, or FCMD) and control humans, and from WT and *Large^{myd}* (*Myd*) mice. Fc-tagged recombinant α -DG was isolated from the

culture medium with protein-A agarose, separated by SDS-polyacrylamide gel electrophoresis, stained with Coomassie brilliant blue (CBB), and analyzed by phosphorimaging (32 P). Phosphorylation of α -DG required prior *O*-mannosylation. Asterisks indicate contaminating proteins derived from fetal bovine serum. (**D**) IMAC-binding assay testing glycoproteins from WT and *Large^{myd}* mice, and from FCMD, MEB, and control human muscle (SkM).

of α -DG. By measuring inorganic phosphate after acid hydrolysis, we confirmed that native α -DG purified from rabbit skeletal muscle is phosphorylated at 4.7 mol of phosphate per mole of protein ($SD = 0.22$, $n = 3$ trials).

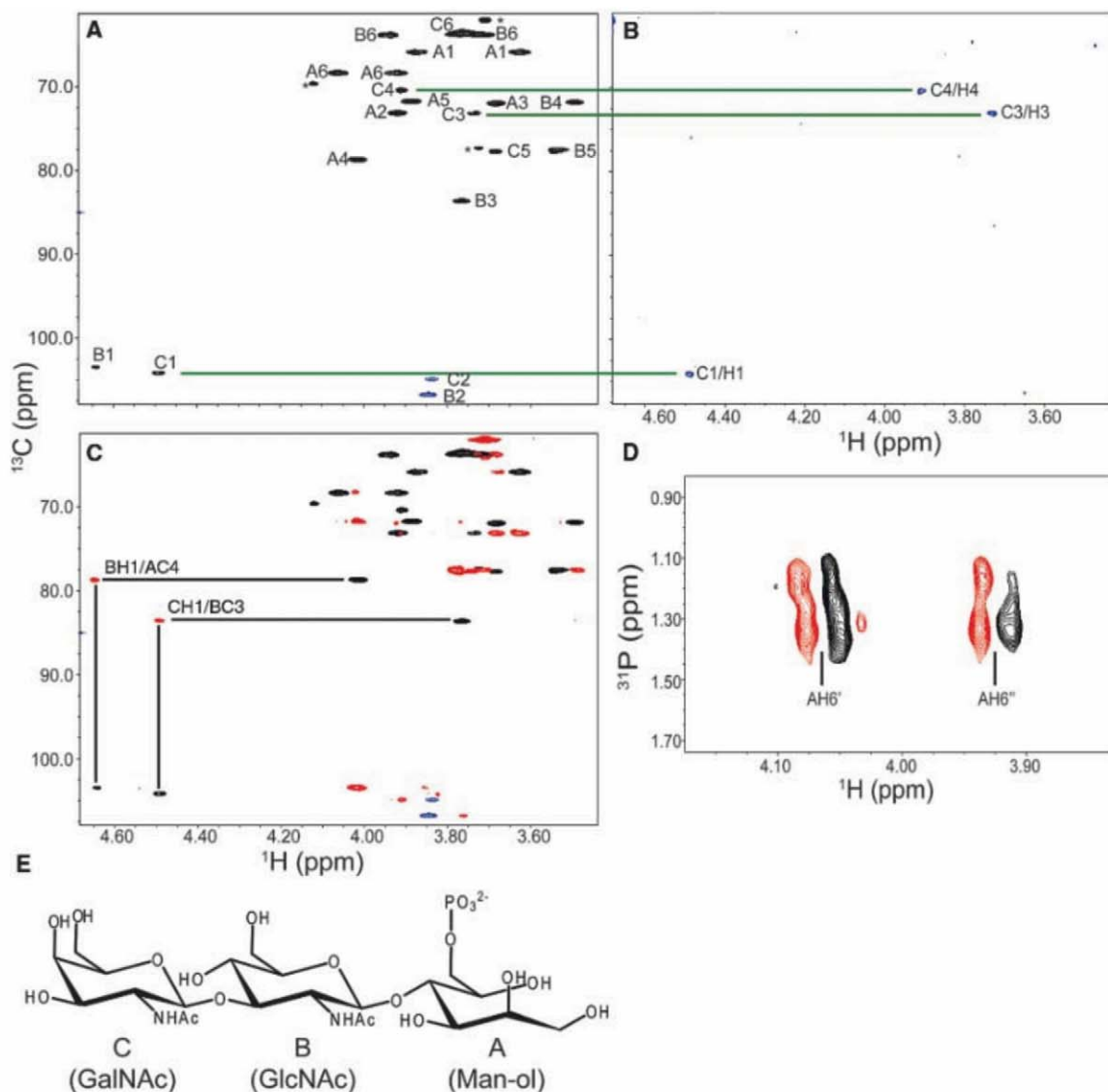
To assess whether CMD cells that synthesize phosphorylated α -DG can further modify the phosphate residue, we immunoprecipitated glycoproteins from mouse *Large^{myd}* and WT muscle, as well as from human *POMGnT1*-mutated MEB, *fukutin*-mutated FCMD, and control muscle, by using immobilized metal affinity chromatography (IMAC) beads that bind to monoester-linked, but not diester-linked, phosphorylated compounds. Only *fukutin*-mutated FCMD and *Large^{myd}* muscle α -DG were captured by the beads, revealing that the phosphate residue does not undergo further modification in these CMD cells (Fig. 2D). This finding suggests that *fukutin* and *LARGE* participate in a common pathway to assemble the laminin-binding moiety onto the phosphorylated *O*-linked mannose. This speculation is compatible with the fact that α -DG prepared from *Large^{myd}*

muscle that was rescued by adenovirus-mediated expression of *LARGE* regains laminin-receptor activity and concomitantly loses its affinity for IMAC beads (fig. S3). In the case of *POMGnT1*-mutated MEB patient muscle, several forms of α -DG were observed; the majority of these were captured by the beads, although a certain amount of α -DG with laminin-binding activity was detected in the void fraction (Fig. 2D). This finding suggests that a defect in *POMGnT1* partially inhibits its modification on the phosphoryl branch chain of the *O*-mannosyl glycan on α -DG.

DGFc4 that was produced by HEK293 cells bound to IMAC beads (fig. S4A) and gained laminin-binding activity when it was coexpressed with *LARGE* (fig. S4B); such a gain in activity has also been observed in FCMD, MEB, and *Large^{myd}* cells, both in this study and elsewhere (22). To determine the structure of the phosphorylated *O*-mannosyl glycan that was necessary to assemble the laminin-binding moiety, we prepared *O*-glycans from HEK293-expressed DGFc4 by reductive β elimination, and we iso-

lated the phosphorylated *O*-glycan using IMAC beads. Linear trap quadrupole (LTQ) mass spectrometry-based analyses detected prominent ions at mass-to-charge ratios $m/z = 667$ ($[M-H]^+$) and $m/z = 333$ ($[M-2H]^{2+}$) that are assigned as a phosphorylated trisaccharide composed of HexNAc₂Hexitol₁ (fig. S5), and analysis by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) revealed the compositional sugars to be GlcNAc, GalNAc, and mannitol (fig. S6). Homo- and heteronuclear NMR techniques were used to assign the $^{13}C/^{1H}$ heteronuclear multiple quantum coherence (HMQC) spectrum of the reduced *O*-glycan (Fig. 3A). The GlcNAc (subunit B) was assigned using double quantum-filtered correlation spectroscopy (DQF-COSY) and total correlation spectroscopy (TOCSY) spectra with a series of mixing times (fig. S7). The GalNAc (subunit C) was partially assigned based on a selective TOCSY-HSQC spectrum (Fig. 3B). The GalNAc (subunit C) is linked via a β 1-3 linkage to the C3 position on GlcNAc (subunit B), which is in turn con-

Fig. 3. NMR analysis of phosphorylated *O*-glycan on HEK293-produced DGFc4. (A) HMQC spectrum where the assigned cross peaks are labeled with a letter for the subunit designated in (E) and a number for the position on that subunit. The folded cross peaks are indicated in blue, and the cross peaks derived from sample impurities are marked by asterisks. (B) TOCSY-HSQC spectrum obtained using a selective excitation pulse at the subunit CH1 proton and a selective TOCSY mixing time of 113 ms. (C) HMBC (red) and HMQC (black and blue) spectra for the assignment of interglycoside linkages. (D) $^{31}P/^{1H}$ COSY spectrum. (E) Structure of the *O*-glycan, with the sugar subunits labeled A to C. ppm, parts per million.



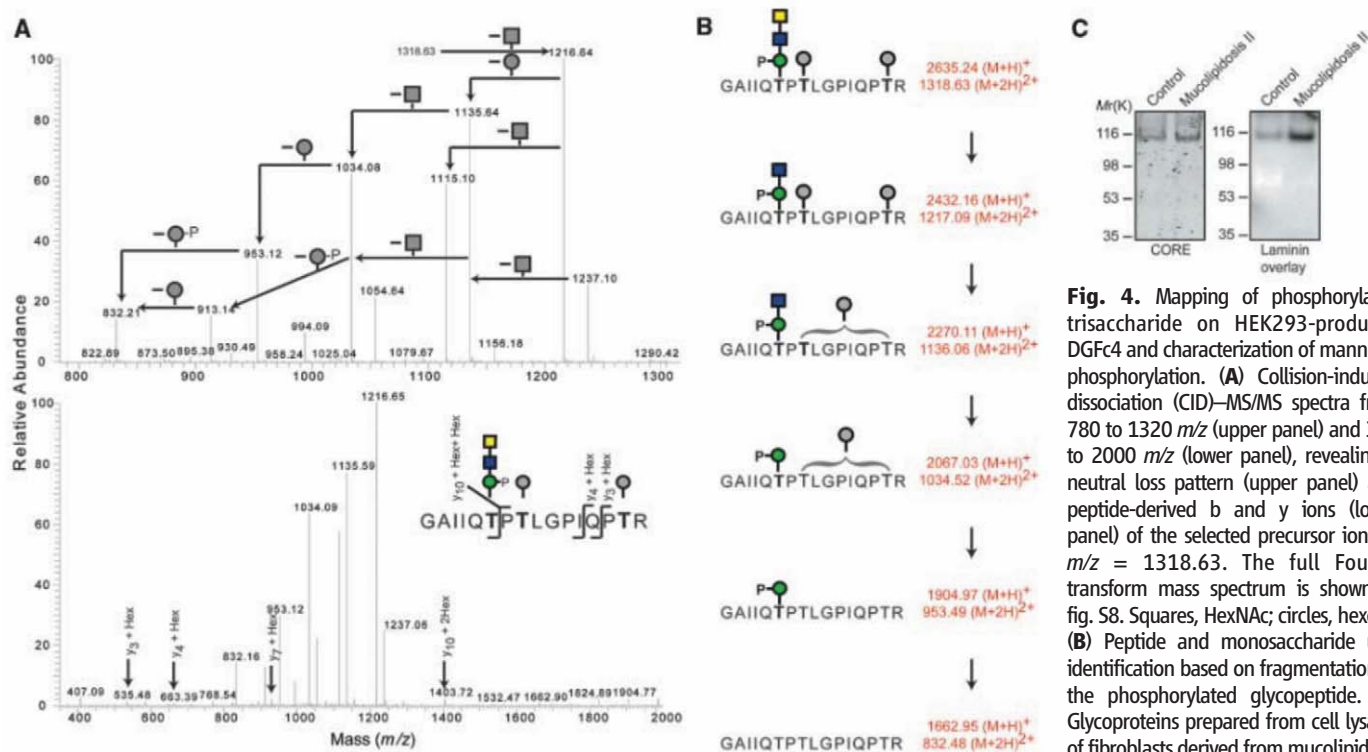


Fig. 4. Mapping of phosphorylated trisaccharide on HEK293-produced DGFc4 and characterization of mannosyl phosphorylation. **(A)** Collision-induced dissociation (CID)–MS/MS spectra from 780 to 1320 m/z (upper panel) and 375 to 2000 m/z (lower panel), revealing a neutral loss pattern (upper panel) and peptide-derived b and y ions (lower panel) of the selected precursor ions at $m/z = 1318.63$. The full Fourier transform mass spectrum is shown in fig. S8. Squares, HexNAc; circles, hexose. **(B)** Peptide and monosaccharide unit identification based on fragmentation of the phosphorylated glycopeptide. **(C)** Glycoproteins prepared from cell lysates of fibroblasts derived from mucopolipidosis II patients were subjected to immunoblotting with CORE antibody and laminin overlay assay.

nected via a β 1-4 linkage to the C4 position on mannitol (subunit A), as evidenced by the observed heteronuclear multiple-bond correlation (HMBC) cross peaks CH1/BC3 and BH1/AC4 (Fig. 3C). The phosphate group is attached to the C6 position of the mannitol (subunit A) as determined from the cross peaks $^{31}\text{P}/\text{AH6}'$ and $^{31}\text{P}/\text{A6H}''$ detected in the $^{31}\text{P}/^1\text{H}$ COSY spectrum (Fig. 3D). The complete NMR resonance assignments of the reduced *O*-glycan and its inter-residue correlations detected in the nuclear Overhauser effect spectroscopy (NOESY) and HMBC spectra are summarized in table S1, and the determined structure is shown in Fig. 3E. To verify that this phosphorylated trisaccharide modifies the mucin-like domain of DGFc4, we enriched the trypsinized peptides using *Wisteria floribunda* agglutinin-lectin and analyzed the GalNAc-terminated peptides by liquid chromatography–mass spectrometry (LC-MS)/MS. MS/MS fragmentation patterns at $m/z = 1318.63$ (Fig. 4A), 1420.17 (fig. S9), and 1501.19 (fig. S10) identified a peptide (amino acids 374 to 389 of α -DG; GenBank ID CAA45732) bearing these modifications: the phosphorylated trisaccharide in conjunction with Hex-HexNAc-Hex, HexNAc-Hex, or Hex. The presence of nonphosphorylated mannose-initiated structures on y3, y4, and y10 ions revealed that Thr³⁷⁹ is modified by the phosphorylated trisaccharide in all cases (Fig. 4, A and B, and figs. S9 and S10). Additional studies showed that α -DG is phosphorylated within the Golgi complex (fig. S11) and that this phosphorylation occurs independently from the

mannose-6-phosphate synthetic pathway that is required for lysosomal protein modification (23); fibroblasts derived from patients with mucopolipidosis II (OMIM ID 252500), which have a defect in GlcNAc-1-phosphotransferase, can synthesize the laminin-binding form of α -DG (Fig. 4C).

We demonstrated that MEB, FCMD, and *Large*^{myd} cells, which have genetically distinct abnormalities, show a similar defect in post-phosphoryl modification on the *O*-mannosyl glycan (fig. S12). These convergent mechanisms to pathology offer an explanation for previous reports that forced expression of LARGE can circumvent defects in α -DG modification in these CMD cells (22). We speculate that LARGE, a putative glycosyltransferase with catalytic domains sharing homology with β 1,3-N-acetylglucosaminyltransferase and bacterial glycosyltransferase (19) participates in post-phosphoryl glycosylation, because the forced expression increases the affinity of the cell surface for both the IIH6 antibody (fig. S13A) and the *Vicia villosa* lectin (fig. S13B). To our knowledge, we provide the first evidence that a vertebrate non-glycosylphosphatidylinositol-anchored glycoprotein is modified by a phosphodiester linkage. Glycoproteins in the cell walls of yeasts and fungi bear phosphodiester-linked glycans that are generated by a process involving phosphorylation on the C6 hydroxyl of mannose (24). α -DG, which is well conserved as an epithelial cell-surface protein in species ranging from lower vertebrates to mammals, is likewise modified by this ancient type of

glycosylation. A recent study has shown that the most severe form of CMD—WWS—is a genetically heterogeneous disease. Moreover, only 40% of WWS cases are explained by mutations in known CMD-causative genes (25). Thus, a defect in the phosphorylation of an *O*-linked mannose may be responsible for severe CMD, indicating that the discovery of mutations in new genes responsible for WWS may not be far off.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5961/88/DC1
Materials and Methods

SOM text
Figs. S1 to S13
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The Rate and Molecular Spectrum of Spontaneous Mutations in *Arabidopsis thaliana*

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To take complete advantage of information on within-species polymorphism and divergence from close relatives, one needs to know the rate and the molecular spectrum of spontaneous mutations. To this end, we have searched for de novo spontaneous mutations in the complete nuclear genomes of five *Arabidopsis thaliana* mutation accumulation lines that had been maintained by single-seed descent for 30 generations. We identified and validated 99 base substitutions and 17 small and large insertions and deletions. Our results imply a spontaneous mutation rate of 7×10^{-9} base substitutions per site per generation, the majority of which are G:C→A:T transitions. We explain this very biased spectrum of base substitution mutations as a result of two main processes: deamination of methylated cytosines and ultraviolet light-induced mutagenesis.

Most of what we know about molecular evolution comes from the comparison of biological sequences that have survived many cycles of natural selection. In order to infer the properties of the original source of variation and to detect the signature of natural selection from such data sets, we need to assume that variants affecting certain types of sites, such as the last base of fourfold redundant codons or pseudogenes, are not subject to natural selection. This pervasive assumption is very rarely tested and difficult to avoid, because of the slow pace of spontaneous mutagenesis. However, with the advent of high-throughput sequencing technologies, some estimates of the rate of spontaneous mutations have begun to appear (1–3). Here, we report a direct estimate of the spontaneous base substitution rate in *Arabidopsis thaliana*, a plant species with extensive DNA methylation. As a result, we reduce the uncertainty associated with key aspects of the evolutionary history of this species, including the time since divergence from *A. lyrata* and the effect of methylation on the probability of mutation.

We sequenced the genomes of five individuals derived by 30 generations of single-

seed descent from the reference strain Col-0 (4), for which a high-quality genome was published in 2000 (5). We used the Illumina (Illumina, San Diego, CA) Genome Analyzer platform to obtain a depth of sequence coverage of between 23 and 31 in each mutation accumulation (MA) line. Sequencing reads between 36 and 43 base pairs (bp) in length were aligned to the reference genome, from which over 1000 errors had been removed in a previous study (6). We identified single-base substitutions using two complementary methods: a “consensus” approach and the single-nucleotide polymorphism (SNP) caller function of SHORE (<http://1001genomes.org/downloads/shore.html>) (7).

In the consensus approach, base substitutions were called if one of the MA lines differed from all others. We estimated a frequency of sequencing errors of ~0.3% per site per read (8). We assumed a binomial distribution of errors to derive the probabilities of false positives and false negatives, and we corrected our estimates accordingly (9). Because sequencing and mapping errors are not randomly distributed among sites (3), we used strict quality filters to exclude from analysis sites suspected to have higher error rates (8). Between 93 and 95 million sites out of the 120 million-bp reference genome matched the quality requirements in each line. Across all five lines, 85 single-base substitutions were called by this method, 83 of which were confirmed by Sanger sequencing. In the other two sites, two or three lines had a nonreference base, whereas the rest matched the reference, and we interpret this to be a result of differential fixation of the two alleles present in ancestrally

heterozygous sites rather than as parallel mutations, although the latter cannot be ruled out.

In addition to this conservative approach, we used SHORE to detect single-base substitutions, short insertions and deletions (indels) of up to 3 bp, and long deletions. The algorithms implemented in SHORE are more sensitive (8), and between 98.8 and 100.9 million sites in each line had sufficient read information for calling either a mutation or the reference base. We detected 99 single-base substitutions (98 of which were confirmed by Sanger sequencing, and 1 was rejected because the reference base was revealed), 9 short deletions (8 confirmed, 1 rejected), 5 short insertions of 1 bp (all confirmed), and 8 long deletions covering 11 to over 5000 bp (4 confirmed, 2 ambiguous, and 2 rejected). A 2-bp deletion was shown to be present in two lines, suggesting that it was heterozygous in the ancestral line. The chromosomal positions of all validated mutations are shown in Fig. 1. Both false positives and false negatives are expected to be absent from the final set of simple base-substitution mutations (8). Fifteen sites where all MA lines had a common composition, but different from the reference, including 13 single-base substitutions and two deletions of 1 and 2 bp, were interpreted as fixed mutations in the ancestral line.

We estimated the overall mutation rate to be $5.9 \times 10^{-9} \pm 0.6 \times 10^{-9}$ base substitutions per site per generation according to the consensus approach and $6.5 \times 10^{-9} \pm 0.7 \times 10^{-9}$ according to SHORE. In addition, joint maximum-likelihood estimates of the overall mutation rate and the sequencing error frequency were obtained following a recently developed method (9). With this approach, a slightly higher mutation rate of $7.1 \times 10^{-9} \pm 0.7 \times 10^{-9}$ at a slightly lower error frequency of 0.2% was estimated. Mutations were evenly distributed among MA lines (Table 1). Within chromosomes, a significantly higher base-substitution mutation rate for intergenic regions was observed closer to the centromere (within 3.0×10^6 bp, for example) than farther away (Fisher's exact test, P value = 0.01, for nonmethylated sites).

The estimated rates of 1- to 3-bp deletions and insertions are $0.6 \times 10^{-9} \pm 0.2 \times 10^{-9}$ and $0.3 \times 10^{-9} \pm 0.1 \times 10^{-9}$ per site per generation, respectively. Out of the 13 short indels that we observed, 6 were found in complex sequences, corresponding to a mutation rate of $4.0 \times 10^{-10} \pm 1.6 \times 10^{-10}$ indels per site per generation, or about 0.05 ± 0.02 indels per haploid genome per generation, excluding homopolymers and microsatellites. This estimate should be considered a

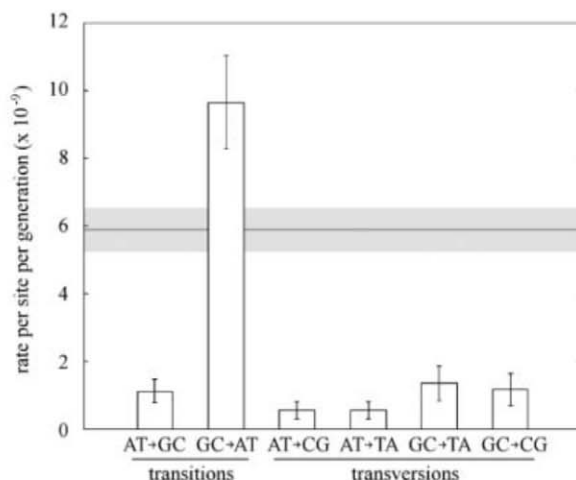
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Fig. 2. Conditional mutation rates per A:T or G:C site per generation. Complementary mutations, such as A→C and T→G, are pooled. Error bars indicate standard errors of the mean. The overall mutation rate, which is the average of the total mutation rates at A:T and G:C sites, and its standard error in gray are shown in the background. Only estimates from the consensus method are shown.



reported to be at least partially methylated had a higher probability of mutation to A:T than non-methylated sites (Fisher's exact test, $P = 3.2 \times 10^{-7}$). However, 91% of G:C sites in *A. thaliana* were not reported to be methylated, and they too had a higher rate of transition (but not transversion) than A:T sites (Fisher's exact test, $P = 1.2 \times 10^{-8}$). G:C sites in CpG contexts are known to be more frequently methylated (20, 21). However, transitions at G:C sites not known to be methylated do not happen in CpG contexts more often than expected by chance (Fisher's exact test, $P = 0.6$). This suggests that factors in addition to methylation contribute to the high rate of transitions at G:C sites.

In both prokaryotes and eukaryotes, most of the mutations caused by ultraviolet (UV) light are G: C→A:T transitions at sites where the C is adjacent to another C or to a T (dipyrimidine sites) (22). Among the 33 observed transition mutations at nonmethylated G:C sites, 31 were in dipyrimidine contexts, which is more than expected by chance at the $P = 0.02$ level (Fisher's exact test). Thus, we conclude that the increased rate of transitions at G:C sites, relative to A:T sites, can be largely explained by the combined effect of UV-induced mutagenesis and deamination of methylated cytosines. This implies that the mutation rate in nature could be higher than that reported here, because UV radiation during the MA experiment was probably lower than in natural conditions.

We used the *Arabidopsis* Information Resource (TAIR) 8 annotation (www.arabidopsis.org) to group all analyzed sites into the functional classes: intergenic, intronic, untranslated region (UTR), coding, pseudogene, mobile element, and non-coding. There is no deficit of nonsynonymous mutations (G test, $P = 0.4$), supporting the notion that the mutation rate we observed is not affected by selection. We did, however, observe an excess of intergenic mutations, relative to mutations in coding regions, introns, and UTRs (fig. S3). These differences were still significant after taking into account the effects of base composition and methylation (G test, $P = 0.00025$). To test whether the lack of mutations in genic regions was due to undetected levels of selection, we compared the intergenic

mutation rate with the rate at synonymous sites and introns, which are less likely to be under strong selection, and we still detected a significant deficit of mutations in the latter (Fisher's exact test, $P = 0.001$, for nonmethylated sites). We attribute the deficit of genic mutations to our observation of a higher mutation rate in pericentromeric regions (see above), where gene density is lower (5), although transcription-coupled DNA repair could also contribute to the pattern. Lastly, the finding of a higher mutation rate in pericentromeric regions provides an explanation of the *Arabidopsis*-specific pattern of higher polymorphism levels near the centromeres (16, 23), although the underlying mechanism of such a mutational bias remains to be explained.

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References

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Targeted 3' Processing of Antisense Transcripts Triggers *Arabidopsis* FLC Chromatin Silencing

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Noncoding RNA is emerging as an important regulator of gene expression in many organisms. We are characterizing RNA-mediated chromatin silencing of the *Arabidopsis* major floral repressor gene, *FLC*. Through suppressor mutagenesis, we identify a requirement for CstF64 and CstF77, two conserved RNA 3'-end-processing factors, in *FLC* silencing. However, *FLC* sense transcript 3' processing is not affected in the mutants. Instead, CstF64 and CstF77 are required for 3' processing of *FLC* antisense transcripts. A specific RNA-binding protein directs their activity to a proximal antisense polyadenylation site. This targeted processing triggers localized histone demethylase activity and results in reduced *FLC* sense transcription. Targeted 3' processing of antisense transcripts may be a common mechanism triggering transcriptional silencing of the corresponding sense gene.

Extensive noncoding RNA has been found in many organisms (1, 2). The degree and mechanism of processing of these transcripts is unclear, as studies of RNA processing

have so far focused on coding (messenger) RNA transcripts in mammalian and yeast cells. The large protein complex mediating 3'-end processing and polyadenylation has recently been de-

scribed (3, 4); however, its role in processing noncoding transcripts is unknown. Analysis of 3' processing of unstable noncoding RNAs has identified independent termination pathways with different components (5).

We have been studying the function of RNA processing in chromatin regulation through analysis of the gene encoding the major *Arabidopsis* floral repressor FLOWERING LOCUS C (*FLC*) (6). The autonomous floral promotion pathway results in *FLC* transcriptional silencing through the activities of two RNA-binding proteins, FCA and FPA; a member of a cleavage and polyadenylation specificity factor (CPSF) RNA 3'-processing complex, FY, which interacts directly with FCA (7, 8); and FLD, a dimethylated histone H3 at lysine 4 (H3K4me2) demethylase and homolog of human LSD1 (9).

To better understand how RNA 3'-processing links to histone modification to result in *FLC* silencing, we sought to identify all the components necessary for FCA-mediated *FLC* repression through suppressor mutagenesis. We generated an *Arabidopsis* line sensitized to FCA action expressing 35S::FCAγ—a transgene overexpressing FCA, *FRIGIDA*—a strong activator of *FLC* expression and *FLC*::LUC, so *FLC* expression could be monitored with a bioluminescence detection assay (10, 11). Mutations that suppressed the ability of FCA to repress *FLC*, together with an epistasis analysis of their interaction with *fca* mutants, enabled us to identify components required for FCA action (fig. S1). These mutations, named suppressors of overexpressed FCA (*sof*), identified additional alleles of known FCA pathway components *FY* and *FLD* (fig. S2) and new complementation groups *sof2* and *sof19* (Fig. 1A and fig. S2). Genetic mapping and sequencing revealed *sof2* and *sof19* carried mutations in *CstF77* (At1g17760) and *CstF64* (At1g71800), respectively (fig. S3, A to C).

CstF77 and *CstF64* are the *Arabidopsis* homologs of two of the three components of the CstF RNA 3'-processing complex, conserved from yeast to plants and humans (12). *CstF77* is a single-copy gene in the *Arabidopsis* genome. In *sof2* (carrying the *cstf77-1* allele), the splice acceptor site in intron 12 is mutated, which leads to an in-frame deletion of Tyr³³⁹ to Lys³⁸⁵ in exon 13 (fig. S3A). *CstF77* is an essential gene in other organisms (13, 14), yet *cstf77-1* plants are viable, but their flowering is delayed. *cstf77-1* is likely to be a hypomorphic allele, because homozygous mutant progeny could not be identified from a mutation caused by a transferred DNA insertion into the 5' end of the gene (*cstf77-2*) (fig. S3A). Further analysis suggested that *cstf77-2* causes female gametophytic lethality (fig. S4, A and B),

consistent with an essential function for *CstF77*. The *sof19* mutation introduces a premature stop codon in the only full-length homolog of *CstF64* in the *Arabidopsis* genome (fig. S3A). This allele (*cstf64-1*) also reduces fertility of the plants. A second, strong allele in Columbia (Col) background (*cstf64-2*) (fig. S3A) leads to reduced organ size, pale leaves, and sterility (fig. S4, C to E). Therefore, *CstF64* function is generally required in plant growth and development. In mammals, *CstF77* interacts physically with *CstF64* through a proline-rich region (15). This interaction was found to be conserved in plants and to be unaffected by the in-frame deletion in *cstf77-1* (fig. S4F).

We further analyzed the involvement of *CstF64* and *CstF77* in *FLC* repression in genotypes not sensitized to FCA action. *cstf64-1*, *cstf64-2*, and *cstf77-1* had elevated *FLC* mRNA levels and flowered later than their respective controls (Fig. 1, B and C, and fig. S5). We also undertook an epistasis analysis to determine whether the CstF components functioned in the same pathway with known FCA pathway components, namely FCA, FY, and FLD. *cstf64-2* was not additive with *fca-9*, *fy-2*, or *fld-4* mutations but was additive with *fve-3* on the basis of analysis of both flowering time and *FLC* expression levels (Fig. 1C and fig. S6). FVE functions to repress *FLC* expression by histone deacetylation in an FCA-independent manner (16). In summary, our analysis demonstrates that CstF components do function in the same pathway as FCA in the repression of *FLC*.

Because the genetic analysis established that CstF components function with the histone demethylase FLD to down-regulate *FLC*, we tested whether the increased *FLC* in *cstf* mutants was an effect of defective transcriptional repression. *cstf64* and *cstf77* mutants showed elevated *FLC* nascent transcript levels (Fig. 2B and fig. S7);

higher RNA polymerase II (Pol II) association at *FLC* (Fig. 2C); and higher levels of H3K4me3, a histone modification associated with active transcription (17) (Fig. 2D). The CstF complex is generally required for canonical mRNA 3'-end formation (12), but its loss did not affect *FLC* sense RNA 3' processing; indeed, *FLC* sense levels accumulate and are functional as evidenced by the late flowering of *cstf64* double mutants (Fig. 1C). Thus, CstF components mediate transcriptional repression of *FLC* levels but are unlikely to be required for *FLC* mRNA 3'-end formation.

FCA directly associates with *FLC* chromatin (9), so one explanation for our observations is that the substrates of CstF64, CstF77, FCA, and FY are other nascent transcripts from the *FLC* locus. We have previously described alternatively polyadenylated *FLC* antisense transcripts (9, 18). One polyadenylation site of the *FLC* antisense RNA coincides with the location of FCA on *FLC* chromatin, whereas a distal polyadenylation site overlaps with the *FLC* sense promoter (9). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis revealed increased levels of *FLC* antisense transcription in *fca*, *fy*, and *cstf* mutants (fig. S8A). Further analysis has also revealed additional complexity in the antisense transcripts, so we developed an assay to detect RNA 3' processing and polyadenylation at the specific polyadenylation sites shown in Fig. 3. qRT-PCR revealed that *FLC* antisense RNA 3' processing was reduced at both proximal and distal polyadenylation sites in *cstf64* and *cstf77* (Fig. 3B), but *FLC* sense RNA polyadenylation was not reduced (fig. S8B). Thus, 3' processing and polyadenylation of *FLC* antisense transcripts, but not *FLC* sense transcripts, appear sensitive to CstF complex activity. Rapid amplification of cDNA 3' ends (RACE) analysis revealed that the same polyadenylation sites are

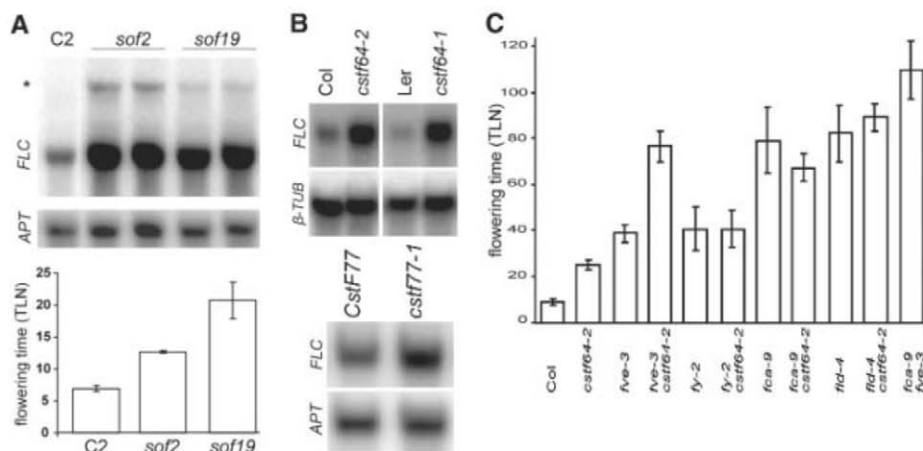


Fig. 1. CstF64 and CstF77 regulate *FLC* levels. (A) Northern and flowering time analysis (total leaf number) of *sof2* (*cstf77-1* in FCA-sensitized background) and *sof19* (*cstf64-1* in FCA-sensitized background). Asterisk indicates *FLC*::LUC; APT is the loading control. (B) Northern analysis of *FLC* levels in *cstf64* carrying no transgenes or *cstf77* carrying the linked *FRI* transgene. β -TUB or APT is the loading control. (C) *cstf64-2* is not additive with *fca-9*, *fy-2*, or *fld-4* (all Col genotype). (A) and (C) graph values are means $\pm$ SD ($n = 20$).

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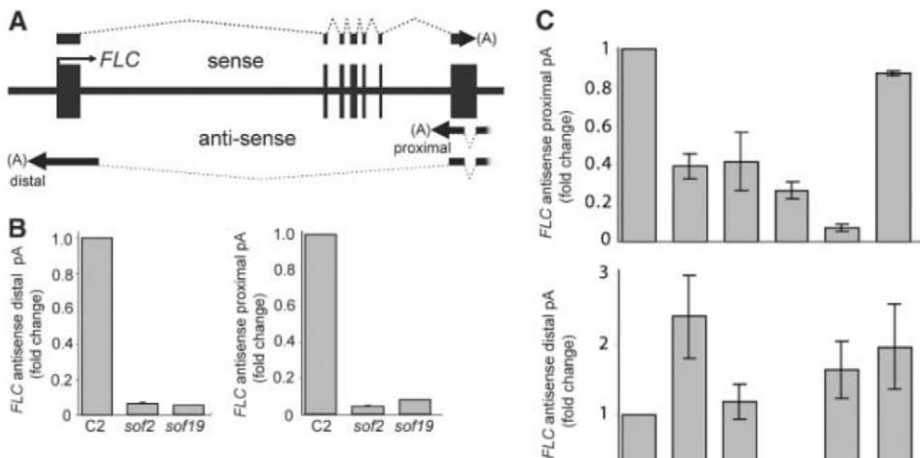
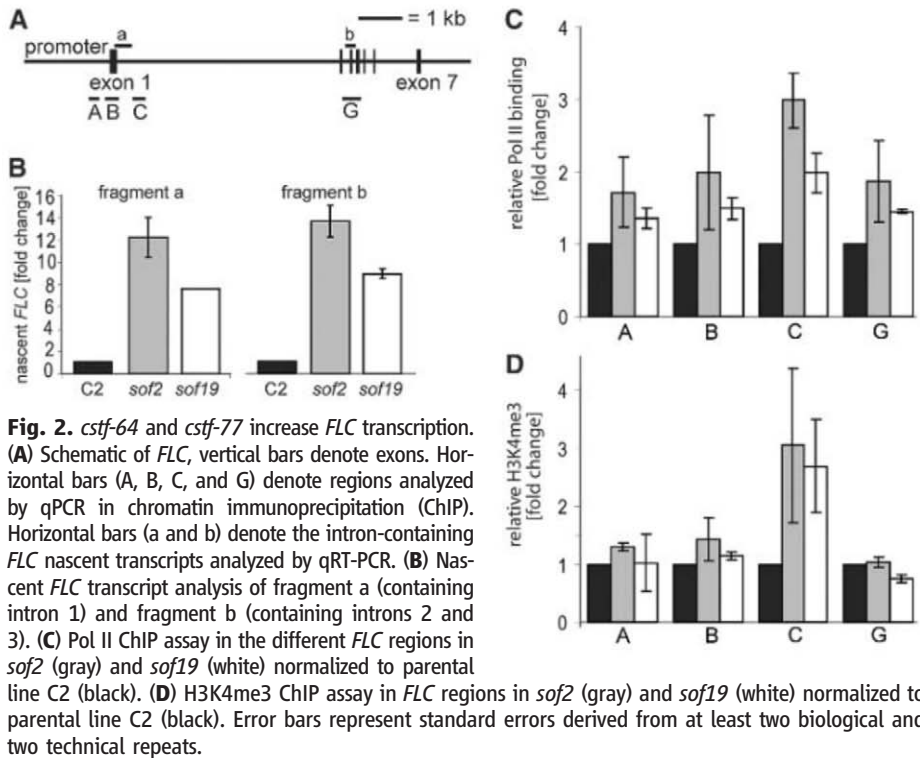
used in the *fca* mutant and wild type; however, qRT-PCR, supported by strand-specific Northern analysis, showed that the relative usage of each site differed. In *fca* and *fy*, proximal site usage was reduced, and in *fca*, distal site usage increased (Fig. 3C and fig. S8C). This is not a feature of general *FLC* de-repression, because

proximal site usage was not affected in *five* (Fig. 3C) (16). To investigate the generality of this finding, we analyzed the effects of FPA, a second RNA recognition motif (RRM) protein that functions in the autonomous floral promotion pathway. FPA also requires FLD to repress *FLC* (19), but it functions independently of FCA (19). As

in *fca*, proximal site usage in the *FLC* antisense transcript was reduced, and distal site usage increased in *fpa-7* (Fig. 3C). Because the two RRM proteins trigger *FLC* silencing independently, we predicted that disruption of FPA function would cause increased *FLC* expression even in the sensitized FCA background. This was confirmed through the identification of *sof34* as a novel *fpa* mutation (fig. S9). Taken together, these results reveal that both RNA-binding proteins independently function to promote 3' processing at the proximal site in the *FLC* antisense transcript and that this activity triggers *FLC* sense strand transcriptional silencing.

The position of the proximal 3'-processing site on the antisense transcript coincides with the site where FCA associates with *FLC* chromatin (9). This suggests a model whereby FCA, interacting with a component of the CPSF complex, targets CstF-dependent 3' processing to the proximal site on *FLC* antisense transcripts (fig. S10). An indirect effect, via CstF regulation of an intermediary regulator, is possible, but unlikely, because of the direct association of FCA with *FLC*. Proximal *FLC* antisense transcript 3' processing is promoted by FCA and FY (Fig. 3), which is reminiscent of the FCA-FY interaction-dependent 3'-processing site choice in *FCA* negative-feedback regulation (7). FPA also enhances usage of this proximal 3'-processing site. These activities then appear to converge to trigger FLD-dependent demethylation of H3K4me2 in the body of the gene, downstream of the proximal polyadenylation site. We speculate that this may involve termination-associated processes, perhaps cotranscriptional decay of the antisense RNA downstream of the cleavage site (20). Whatever the mechanism, down-regulation of both sense and antisense transcription is the net result. We suspect the activity of DICER-LIKE3, previously shown to function in this process (9), is required to couple the 3' processing to FLD histone demethylase function (21); however, RNA polymerases involved in silencing mediated by small interfering RNA (siRNA) (18) only weakly suppress FCA activity (fig. S11). A role for small RNAs in the targeting of the histone demethylase activity to the central part of the locus may lead to *trans* effects on homologous sequences in the genome, perhaps explaining the small effect of an *fca* mutation on an *FLC* transgene lacking the 3' region (22).

The proteins involved in this silencing mechanism—two RRM proteins, a factor associated with 3' processing components, and a conserved histone demethylase—also silence transposons and transgenes in *Arabidopsis* (20, 23). This mechanism may therefore play a more general role, rather than only regulating the floral repressor gene, *FLC*. This is consistent with the role for CPSF complex components in suppressing viral amplicon-induced gene silencing in *Arabidopsis* (24). This mechanism may also be very important in nonplant genomes. Genome-wide antisense transcripts, identified in



many organisms, function in chromatin silencing (2, 25, 26) and RNA 3'-processing components mediate gene silencing in *Caenorhabditis elegans* (27). Therefore, the 3' processing of antisense transcripts may be a general mechanism triggering chromatin silencing in eukaryotes.

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Materials and Methods

SOM Text

Figs. S1 to S11

Table S1

References

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Reproducibility Distinguishes Conscious from Nonconscious Neural Representations

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What qualifies a neural representation for a role in subjective experience? Previous evidence suggests that the duration and intensity of the neural response to a sensory stimulus are factors. We introduce another attribute—the reproducibility of a pattern of neural activity across different episodes—that predicts specific and measurable differences between conscious and nonconscious neural representations independently of duration and intensity. We found that conscious neural activation patterns are relatively reproducible when compared with nonconscious neural activation patterns corresponding to the same perceptual content. This is not adequately explained by a difference in signal-to-noise ratio.

Though once controversial, it is now widely accepted that sensory-perceptual information can be processed by the brain, even at the semantic level, without that information “reaching” or “entering” awareness (1–3). But what does it mean for neural information to reach awareness? Once the information has been encoded in neural activity, what else has to happen for it to become part of one’s subjective reality? A growing body of evidence suggests that the intensity of activation in areas that encode the contents of perception (such as the ventral-temporal cortex) is one determinant of whether or not that information contributes directly to subjective experience (4–7). However, local enhancement of a cortical sensory signal is also associated with attention (8), which can be independent of awareness (9–11). Therefore, there may be additional features other than the intensity of neural activity that distinguish conscious from nonconscious neural information.

Kinsbourne (12) proposes three interacting properties that collectively determine whether or not a neural representation will contribute directly to subjective experience: (i) the duration and (ii) the intensity of a pattern of activity and (iii) the coherence of that pattern of activity with the dominant “configuration” of neural activity at the global level. Here, we propose that another attribute of neural activity patterns, reproducibility, characterizes conscious representations. We define reproducibility as the similarity of patterns of neural activity across different instances of the same percept. We focused specifically on reproducibility because it is measurable and therefore empirically testable. A corollary of our proposal that conscious representations are more reproducible is that unconscious representations are more variable, even as they may carry information within a given episode.

We used functional magnetic resonance imaging (fMRI) to measure brain activity while subjects performed a simple visual category-discrimination task ($n = 12$ subjects) (13). Stimuli were simple line drawings of faces and houses (12 of each), rendered in two opposing but isoluminant colors (Fig. 1) (13). Visibility of

the stimuli was manipulated by using dichoptic color masking (DCM) (Fig. 1) (7). Subjects were asked to identify the category of the stimulus (face or house) on each trial, guessing if necessary, and to wager (“high” or “low” for monetary rewards) on the accuracy of each of their perceptual decisions (14–16). Wagering was used as a collateral index of subjects’ awareness of the object.

For visible stimuli, performance was at or near 100% correct for all 12 subjects, and all wagers were high. For invisible stimuli, task performance was only marginally different from chance ($54 \pm 2.5_{\text{[SEM]}}\%$ correct; $P < 0.06$, one-tailed t test), and sensitivity of high wagers to correct responses [wagering d' -prime, or d' (13)] was not different from zero (mean $d' = 0.015 \pm 0.11_{\text{[SEM]}}$; $P = 0.45$, one-tailed t test). For invisible stimuli, wagering d' and overall willingness to place high wagers were not significantly correlated across subjects [correlation coefficient (r) = 0.33, $P > 0.30$, $n = 12$ subjects]. This reassures against the possibility that wagering d' was artificially low because of an interaction with a wagering bias (16). The proportion of high wagers (for invisible stimuli) was similar for faces and houses (0.20 and 0.19, respectively).

Subjects were always aware of a visual event—a yellowish flickering square—and this provoked substantial activation in and of itself. What varied was subjects’ awareness of an object embedded in the square. We used multivariate pattern analysis to ascertain how the encoding of perceptual information differs depending on whether or not that information is present in subjective experience (17). Thus, in our analyses we focused specifically on the patterns of activation corresponding to the perceptual information of which the subject was or was not aware: the category of the object.

To verify the neural representation of category-specific information for both visible and invisible stimuli, we attempted to discriminate the category of the stimulus (faces versus houses) on the basis of the spatial pattern of neural activity in the temporal lobes [derived statistically from each run of

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functional data (13)]. We did this independently for the visible and invisible stimuli using a Gaussian naïve Bayes (GNB) classifier (18). We focused our analyses on the temporal lobes because these are widely viewed as being critical for high-level perceptual representation of visual information (19). Mean accuracy of the classifier (percent correct averaged across 12 subjects) was significantly different from chance (50%) for both visible [63% correct; Student's *t* test, *t* = 3.82, *P* < 0.002] and invisible (58% correct; *t* = 2.53, *P* < 0.02) stimuli (Table 1). The difference in accuracy for visible versus invisible stimuli was not significant (*P* < 0.2, one-tailed paired-samples *t* test). It might be expected that as long as the classifier performed above chance on both types of stimuli, then it would also perform well when trained on one type and tested on the other (20). However, this was not the case for these stimuli (Table 1).

Each round of training and testing of the classifier involved a dimensionality-reduction step, in which we determined which voxels (features) varied most consistently as a function of stimulus category (feature selection) separately for visible and invisible stimuli (13). Training and testing of the classifier was then performed on these smaller feature spaces ("selections"). Our approach involved the examination of the patterns of activity within these selections of voxels on the assumption that these would reveal properties of information encoding under conditions of conscious and nonconscious perception.

Treating patterns of activation as vectors allows us to test hypotheses about the properties of neural information independently of specific loci and their level of activity. The angle between two activation vectors reflects differences in the contents of perception, whereas the norm of each vector corresponds to the intensity of the information being encoded. We can then define reproducibility as the similarity in the pattern of activity across different instances of the same stimulus category among voxels that carry relevant information. This can be measured by computing the trial-to-trial variability of the vector angle in the space of the voxels selected as informative for classification.

We predicted that activation vectors associated with conscious perception (visible stimuli) would exhibit less trial-to-trial variability in their angle than those associated with nonconscious perception (reflecting greater reproducibility) without necessarily any difference in the norm (that is, in intensity). To assess the reproducibility of representations, we measured the variability in the angle between pairs of vectors (both from the same run and same stimulus category), as well as the norm of each vector, separately for visible and invisible stimuli (13, 21). We repeated this in both the visible and the invisible selections (22). This resulted in four sets of data: responses to visible and invisible stimuli in the visible selection and responses to visible and invisible stimuli in the invisible selection. To avoid confounds that were likely to arise from comparing properties of vectors in different subsets of voxels (and hence different regions of cortex), we restricted

our comparisons to vectors within the same selection (23). We used the mean within-category within-run angular deviation as an index of reproducibility.

Within the invisible selection, the variability of the vector angle (dVA) is significantly less for visible than for invisible stimuli (*P* < 0.01, paired-samples two-sided signed rank test) (Fig. 2B). There was no difference in dVA between visible and invisible stimuli in the visible selection (Fig. 2A), suggesting that the variability is found primarily in the subset of voxels that carry nonconscious information and that this subset is distinct from that within which conscious information is found (for this particular combination of stimuli and task). This is consistent with the failure of the classifier to generalize across the two levels of visibility. When dVA for the invisible selection was compared with the baseline level 4 s earlier (at the time of stimulus onset), there was a significant interaction (*P* = 0.021, two-sided signed rank test on the deviation from baseline): dVA was below baseline in response to visible stimuli and higher than baseline in response to invisible stimuli (Fig. 2B). There was no difference in the mean or variance of the vector norm for visible versus invisible stimuli, either in the visible or invisible selection (means, *P* > 0.35, paired-samples two-sided signed rank test; variances, *P* > 0.7, Levene's test) (Fig. 2, C and D). Thus, a difference in signal-to-noise ratio is not sufficient to explain the effect.

Because measurable category-specific information had been identified separately for both visible and invisible stimuli, we examined where in the

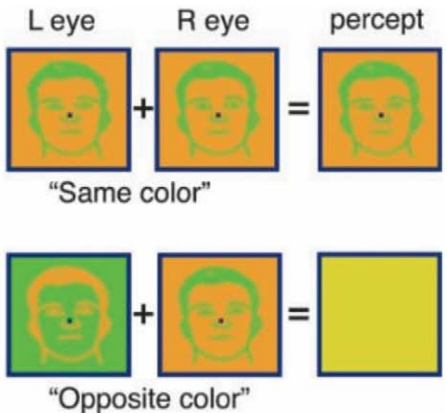


Fig. 1. Dichoptic-color masking. This method of manipulating awareness, originally devised by (7), relies on the phenomenon of dichoptic color fusion. The "same color" mode corresponds to the visible condition, and the "opposite color" mode corresponds to the invisible condition. In order to achieve disappearance of the image in the opposite color mode, the two colors must be approximately isoluminant and the object boundaries slightly blurred. Before the experiment, subjects were trained to maintain steady fixation and were cued to do so during each trial with the appearance of the fixation point (500 ms before stimulus onset). Stimuli were presented stereoscopically in the fMRI scanner by using a cardboard divider and prism lenses (28).

brain the information tended to coalesce in each case (Fig. 3). For any given subject, reliably informative voxels could be found throughout the temporal lobes (Fig. 3A). Averaging across subjects (24) revealed two clusters in the right ventral temporal cortex, one for visible and the other for invisible stimuli, with minimal spatial overlap, which is consistent with the failure of the classifier trained on one type of stimulus to generalize to the other (Fig. 3, B and C). The anterior-posterior relationship of the two clusters (visible and invisible selections, respectively) coincides with previous observations (25).

Conscious and nonconscious neural activation patterns coexist within the cerebral cortex, side by side at the same time, but presumably they differ in several ways. Proposed differences include duration, intensity, and coherence. Here, we show that they also differ in their relative reproducibility across presentations of similar stimuli. Why might reproducibility distinguish conscious from nonconscious representations? One possibility is that conscious information is represented in a more discrete form (26), making it more durable and robust, but also more stereotypical (and therefore more reproducible). Another possibility is that conscious information manifests itself in relatively stable neural firing patterns, corresponding to the "settled" states of recurrent network interactions (27). There are a number of plausible theories regarding the neural correlates of consciousness but relatively little data concerning the nature of conscious versus nonconscious encoding. Further work is required to understand the difference (or differences) in the way perceptual information is encoded in the brain depending on whether or not that information is present in subjective experience. Such work is likely to have profound importance in a variety of arenas, including the assessment of consciousness under presumed

Table 1. Performance of a GNB classifier. The objective of the classifier was to discriminate the category of the stimulus based on the pattern of beta weights [a general linear model (GLM) was applied separately to each run of functional data (13)]. A voxel-wise analysis of variance and nested cross-validation (18) were used for dimensionality reduction on each round of training and testing. For within-condition classification (visible-visible and invisible-invisible) a leave-one-run-out cross-validation was performed. For between-condition classification, we trained on all the data from one condition and tested on the other and vice-versa. All *t* tests are one-tailed with *df* = 11.

Train	Test	
	Visible	Invisible
Visible	63 ± 3.5	48 ± 2.3
	<i>t</i> = 3.8,	<i>t</i> = -0.78,
	<i>P</i> < 0.002*	<i>P</i> = 0.77
Invisible	52 ± 3.0	58 ± 3.1
	<i>t</i> = 0.69,	<i>t</i> = 2.5,
	<i>P</i> = 0.25	<i>P</i> < 0.02*

*Statistically significant; *P* < 0.05.

Fig. 2. Variability in the angle of activation vectors in the visible and invisible selections (**A** and **B**) and mean vector norm (**C** and **D**). In both (**A**) and (**B**), t_0 corresponds to the TR (2 s) on which the stimulus was presented before the hemodynamic response had begun to rise. t_2 corresponds to 2 TRs (4 s) after the stimulus was presented at the (approximate) peak of the hemodynamic response ($n = 12$ subjects). This analysis was performed by using a leave-one-run-out procedure: Voxel selection was performed on data from $n - 1$ runs, and the norm and angular deviation were computed on data from the run that had been left out (13). Comparisons between the two selections [(A) versus (B) or (C) versus (D)] are not valid (23).

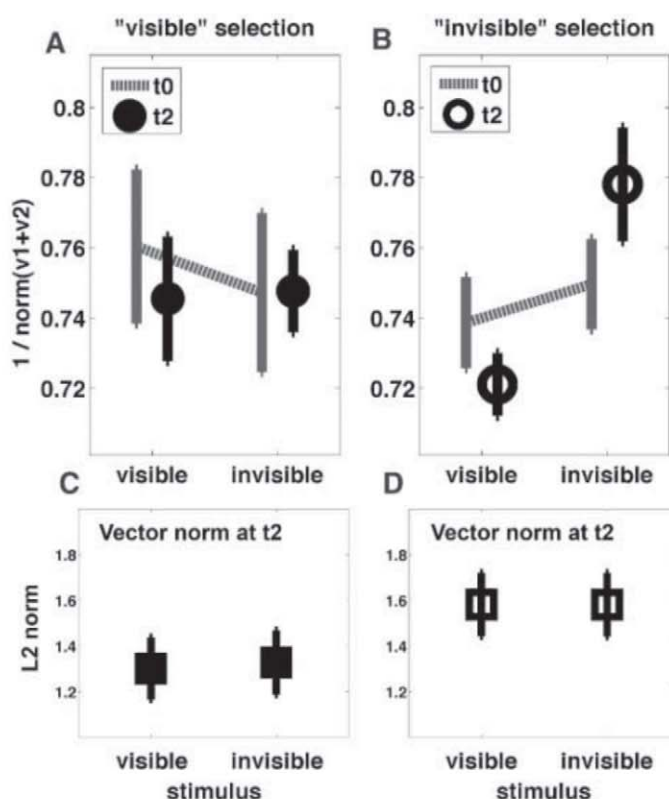
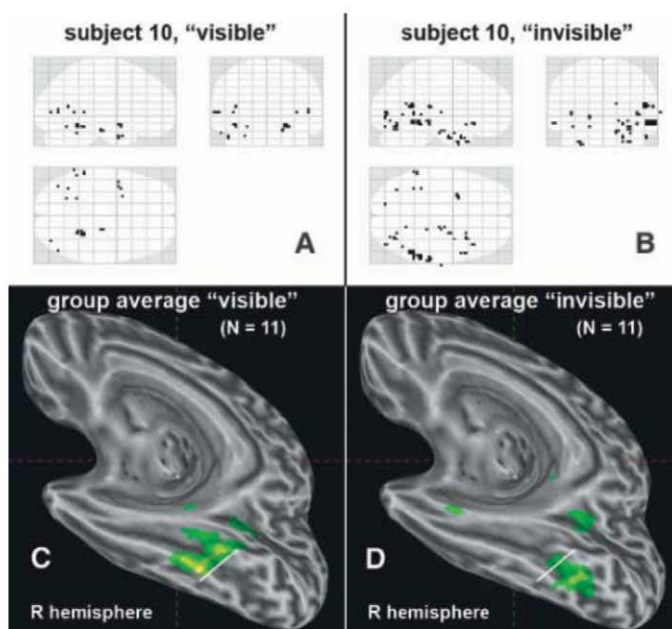


Fig. 3. Spatial distribution of informative voxels. (**A** and **B**) Voxels that were selected as informative (face versus house) on 6 or more (out of 12) runs for a subject with comparable classification accuracy (72% correct) for visible and invisible stimuli. (**C** and **D**) The mean across subjects (24) projected onto the AFNI TT_N27 template brain (right hemisphere) at a statistical threshold of $P < 0.05$ (corrected). The oblique white line serves as a visual landmark. The cluster in (**C**) encompasses a portion of the fusiform and parahippocampal gyri in the area of the fusiform face area (FFA) and parahippocampal place area (PPA). The cluster in (**D**) lies along the posterior fusiform gyrus.



anesthesia or coma and the investigation of brain function in conditions such as schizophrenia, autism, and dissociation disorders.

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21. While voxel selection was based on coefficients derived statistically from each functional run (13), the activation patterns among these voxels were taken trial by trial from the minimally processed fMRI signal data [at $t_0 + 2TR$, where t_0 is time of stimulus onset and 1 TR (time of repetition) = 2 s]. This was done in a leave-one-run-out fashion: The selection was chosen on the basis of data from $n - 1$ runs, and then the activity vectors from the left-out run (2 visible/invisible $\times$ face/house per run) were projected into that space (13).
22. The visible selection comprises the voxels that were maximally informative as to the category of visible stimuli. Likewise, the invisible selection comprises the voxels that were maximally informative as to the category of invisible stimuli.
23. Because the visible selection and the invisible selection occupy separate and largely non-overlapping regions of cortex, comparisons between their functional properties are confounded with differences between the hemodynamic and magnetic-field properties of the regions they inhabit.
24. To produce spatial maps of reliably informative voxels, each voxel was coded with either a 1, if selected on a majority of runs, or a 0 otherwise (Fig. 3, A and B). In order to uncover regional tendencies in the average across subjects, maps for each subject were blurred by ~ 10 mm and then discretized again (ceiling). The probability distribution of the average map under the null hypothesis was estimated by using a permutation test (number of voxels held constant for each subject per selection, but locations randomized) and used to set a statistical threshold.
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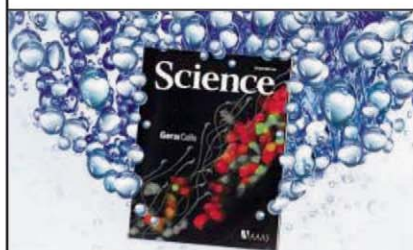
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The Medical College of Georgia is seeking an investigator with outstanding research accomplishments and potential in neuropharmacology and neuroscience to complement and enhance the diversity of basic and clinical neuroscience research strengths in our Department and Institution. MCG has an eminent, well-funded neuroscience research community across the Departments of Pharmacology and Toxicology, Physiology, Cell Biology, Molecular Medicine, Psychiatry and Health Behavior, and Neurology. Faculty members in pharmacology and toxicology conduct research that is relevant to a range of neurological and psychiatric disorders including Alzheimer's disease, Parkinson's disease, drug abuse, schizophrenia, and mental retardation. We seek applicants with a Ph.D. or M.D. degree with a record of productivity and extramural funding. Applicants with expertise in cell signaling, drug discovery, systems neurobiology, or neurotoxicology are particularly encouraged to apply. Strong consideration will be given to applicants with a clear collaborative potential with current faculty members in the Department of Pharmacology and Toxicology. We offer a competitive salary and startup package commensurate with the level of appointment, excellent laboratory space and outstanding core facilities for microarray technology, genetically modified animals, cell imaging, electron microscopy, small animal and nonhuman primate behavior, and clinical collaborations. The successful applicant will participate in teaching programs for professional and graduate students. Please send curriculum vitae, summary of professional and research goals, and the names and addresses of three references to: **Search Committee, c/o Dr. Alvin V. Terry, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300; e-mail: aterry@mail.mcg.edu**. Visit the department homepage at **website: <http://www.mcg.edu/SOM/phmtox/index.html>**. Application review will begin January 15, 2010. MCG is an Equal Employment Opportunity/Affirmative Action/Equal Access Employer. ACH56836



TENURE-TRACK FACULTY POSITION

Assistant Professor in Structural Bioinformatics/Computational Structural Biology

The College of Letters, Arts and Science of the University of Southern California invites applications for a tenure-track faculty position at the level of Assistant Professor, beginning fall 2010. Candidates in all areas of structural bioinformatics and computational structural biology are welcome to apply. The individual will assist in further strengthening and expanding the existing strong computational biology and bioinformatics program. The position is in the interdisciplinary Program in Molecular and Computational Biology (MCB) in the Department of Biological Sciences. The successful candidate will be expected to participate in undergraduate and graduate teaching and to establish a vigorous, externally funded research program. Review of applications will begin immediately. Interested candidates should electronically send curriculum vitae and research plans and should arrange for three letters of reference to be sent to **e-mail: csearch@college.usc.edu** or, if necessary, **Eleni Yokas, Computational Biology Search Committee, Department of Biological Sciences, RRI201, University of Southern California, Los Angeles, CA 90089-2910**. For additional information about our program, visit our **website: <http://www.cmb.usc.edu/>**.

USC strongly values diversity and is committed to equal opportunity in employment. Women and men, and members of all racial and ethnic groups, are encouraged to apply.

Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH



Director of Chemistry, Therapeutics for Rare and Neglected Diseases (TRND) Program National Human Genome Research Institute

The National Institutes of Health (NIH) is seeking a senior scientist to serve as the Director of Chemistry for the Therapeutics for Rare and Neglected Diseases (TRND) Program, a new effort administered by the National Human Genome Research Institute (NHGRI) and the NIH Office of Rare Diseases Research (ORDR). The goal of this Program is to discover and develop small molecules that are suitable for testing in humans and promising for treating individuals with rare and neglected diseases.

As an integral part of the TRND executive team, the Director of Chemistry will provide input on the overall strategy for TRND and lead the research operations of all functions of the Chemistry Section. The successful candidate will be a recognized leader with a proven track record in human drug development, with at least 15 years of relevant synthetic and analytical chemistry experience in the pharmaceutical industry. The candidate must have a Ph.D. in synthetic or medicinal chemistry, an excellent publication record and patent portfolio, should show fluency in structural and computational chemistry, and be cognizant of relevant state-of-the-art techniques and the utilization of specialized technical equipment. The candidate will have demonstrated ability to supervise a large group of chemists effectively and a history of providing outstanding mentorship. The candidate is also expected to be well versed in biology, pharmacology, drug metabolism and pharmacokinetics, as well as have a demonstrated history of effective and successful collaborations.

The TRND Program includes ongoing support for the Director of Chemistry and the scientific team that the Director is expected to build. Interested applicants should send their curriculum vitae, a three-page statement of interest in (and vision for) the Chemistry Section of TRND, and three letters of recommendation through our online application system, at <http://research.nhgri.nih.gov/apply>.

Applications will be reviewed starting January 31, 2010 and will be accepted until the position is filled.

Specific questions regarding the recruitment may be directed to Dr. Carole Bewley, Search Chair, at CaroleB@intr.niddk.nih.gov. Questions may also be directed to Dr. Melissa Ashlock at ashlockma@mail.nih.gov.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.

NATIONAL HUMAN GENOME RESEARCH INSTITUTE Division of Intramural Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR



Department of Health and Human Services National Institutes of Health National Cancer Institute, Center for Cancer Research

Postdoctoral Fellowship positions at the National Cancer Institute

Postdoctoral fellowship positions are available in the Lung Carcinoma Section, Medical Oncology Branch at the National Cancer Institute (NCI), Bethesda, MD. Projects involve studying molecular pathways required to support oncogenesis, identifying new therapeutic targets for Ras-driven cancers and developing new RNAi tools for mammalian genetics. Applicants must have a PhD or MD degree and less than five years' postdoctoral experience. Candidates should have a strong background in molecular biology, genetics or biochemistry, and a strong interest in cancer biology and translational research. For further information about the MOB, NIH or NCI programs, faculty and training, please visit our respective Web sites: <http://ccr.cancer.gov/labs/lab.asp?labid=753>, <http://ccr.nci.nih.gov>, <http://www.nih.gov>. Anticipated starting date for this position is summer 2010. Candidates should e-mail a cover letter, CV, including publications in peer reviewed journals, and the names/phone numbers of three references to:

Dr. Ji Luo

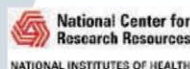
c/o Allyson Cole: colea@mail.nih.gov

Clinical Research ARC, OM, NCI

10 Center Drive, Bldg. 10, Room 12N226

Bethesda, MD 20892-1904

DHHS, NIH and NCI are Equal Opportunity Employers



Division of Construction/ARRA Construction Team Health Scientist Administrator

The National Center for Research Resources, a component of the National Institutes of Health, has been charged by the President of the United States to administer a \$1 billion program under the American Recovery and Reinvestment Act (ARRA) to provide funding for construction, renovation, and/or repair of facilities used for biomedical and behavioral research. In order to accomplish this goal, NCRR has created a Division of Construction to oversee and administer grant awards along with other activities required to ensure the transparency and accountability in the program.

The ARRA construction team will be reviewing construction designs, overseeing the progress of construction, and reporting the status of the program to a variety of audiences, including all levels of Federal Government and the American taxpayers. At full strength, the Division will have 7 members and additional advertisements for a construction project manager, professional engineers, and a program analyst will be announced soon. Please check the job opportunities listed at the NCRR website, www.ncrr.nih.gov, for these specific announcements.

As part of the team, NCRR is currently seeking applications for Health Scientist Administrators. The salary range is \$73,100 to \$133,543 per annum, commensurate with qualifications and professional experience. A full benefits package is available, which includes retirement, Thrift Savings Plan participation, health, life, and long-term care insurance.

For qualification requirements, evaluation criteria, and application instructions, view the vacancy announcements at <http://www.jobs.nih.gov/hsa/>. Please contact **Michelle Lipinski** at 301-594-2286 if you have any questions.



WWW.NIH.GOV

Clinical Tenure-Track Position Division of Intramural Research (DIR)

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR), is seeking an outstanding tenure-track investigator to develop a clinical research program to better understand, treat, and ultimately prevent infectious, immunologic, and/or allergic diseases. The successful candidate will implement and direct an independent clinical research program with an emphasis on clinical studies but which may include translational and basic research. The incumbent can choose the laboratory with which he or she would prefer to be affiliated. Any clinical protocols developed should complement the research goals of the selected laboratory. In addition, the incumbent will be paired with a senior investigator, who will serve as a clinical mentor.

An outstanding postdoctoral record of research accomplishment and an M.D., M.D./Ph.D., or equivalent degree is required for this position; board eligibility/board certification is also required. The incumbent will be expected to meet the requirements for authorization of patient care privileges by the Credentialing Services of the NIH Clinical Center.

Candidates will be assigned independent resources to include clinical and/or laboratory support personnel, equipment, space, and an allocated annual budget for services, supplies, and salaries sufficient to foster success.

This is a tenure-track appointment under Title 42. Salary is dependent on experience and qualifications.

Interested candidates may contact Dr. Karyl Barron, DIR deputy director at 301-496-3006 or kbarron@nih.gov for additional information about the position.

To apply for the position, e-mail your curriculum vitae, bibliography, and an outline of your proposed research program (no more than two pages), by **January 14, 2010** to Ms. Yushekia Hill at NIAID.DIR.Search@niaid.nih.gov.

In addition, send three letters of recommendation to Chair, NIAID DIR Clinical Tenure Track Search Committee, c/o Ms. Yushekia Hill at NIAID.DIR.Search@niaid.nih.gov or 10 Center Drive MSC 1356, Building 10, Room 4A-22, Bethesda, MD 20892-1356. E-mail is preferred. Please note search #027 when sending materials.

National Institute of Allergy and Infectious Diseases

Further information about DIR laboratories is available at www.niaid.nih.gov/about/organization/dir and information about working at NIAID is available at www.niaid.nih.gov/careers/sctt.



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



National Institute of Allergy and Infectious Diseases
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STANFORD UNIVERSITY
SCHOOL OF MEDICINE



LUCILE PACKARD
CHILDREN'S HOSPITAL AT STANFORD

**CENTER FOR EXCELLENCE IN
PULMONARY BIOLOGY**
*Divisions of Pulmonary, Asthma, and
Critical Care Medicine*

**Stanford University
Pediatric Pulmonary
Center of Excellence in Pulmonary Biology**

The newly established Center for Excellence in the Department of Pediatrics at Stanford University seeks an outstanding faculty (UTL) member in Pediatric Pulmonary Medicine.

Essential Requirement for UTL candidates: Be an MD with the Board Certification in Pediatrics, and be Board Eligible or Board Certified in Pediatric Pulmonary Medicine, have medical licensure in California by starting date, and a record of accomplishment in investigator-initiated, hypothesis-driven research.

It is anticipated that the successful faculty candidate will be an excellent researcher, committed teacher, and an outstanding clinician and have an emerging regional/national reputation in Pediatric Pulmonary related research, and have the skills to establish an investigator-initiated research program focused primarily upon discoveries that can be used to inform patient care. The specific focus of the research program will be on translating the clinical observation and laboratory based discoveries into clinical programs.

The overriding requirement for faculty appointment, reappointment and promotion within the UTL must be distinguished performance, or (in the case of junior faculty) the promise of distinguished performance. There should be a major commitment to research and teaching. There must be outstanding accomplishments in research and excellent overall performance in teaching, as well as in clinical care and institutional service appropriate to the programmatic need the individual is expected to fulfill. Such programmatic need, including financial viability, should be evaluated and must be established for each appointment, reappointment and promotion.

Contingent on professional accomplishment, the candidate will be appointed as an Assistant or Associate Professor (University Tenure Line) in the Stanford University School of Medicine. Rank and salary will be commensurate with qualifications and experience.

Applications will be reviewed beginning **July 1st, 2009** and accepted until position is filled. Submit applications and CV to:

David Cornfield, M.D.
Chair, Search Committee
The Anne T. and Robert M. Bass
Professor of Pulmonary Medicine
Director – Center for Excellence in Pulmonary Biology
Service Chief of Pulmonary, Allergy and
Critical Care Medicine
Suite 350, 770 Welch Road
Stanford, California, 94304
dcornfield.edu

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups as well as others who would bring additional dimensions to the university's research, teaching, and clinical missions.



**Iowa Center for Muscular Dystrophy Research and Department
of Molecular Physiology and Biophysics**
University of Iowa Carver College of Medicine

Faculty Positions
Skeletal Muscle Biology and Disease

The Iowa Center for Muscular Dystrophy Research seeks outstanding candidates for tenure-track positions at any rank. Successful candidates are expected to establish independent laboratories focusing on skeletal muscle biology and disease. We are particularly interested in individuals who would complement existing strengths in the Center.

These positions feature outstanding research space with state-of-the-art shared instrumentation, as well as substantial startup funds for equipment, personnel support and supplies. The University of Iowa is located in Iowa City, an affordable college community with many cultural amenities.

All applicants must have a relevant doctoral degree, productive research experience, and a strong record of research accomplishment. Candidates are judged on their potential to initiate and maintain a creative, independent research program, and their desire to train students and postdoctoral fellows.

To apply for this position, please visit the University of Iowa website at <http://jobs.uiowa.edu/faculty/view/57388>. Applicants should include a CV, letter of interest, and the names of three references. Consideration of completed applications will begin on **February 15, 2010**. Questions may be directed to **Dr. Kevin P. Campbell, Director of the ICMDR** at kevin-campbell@uiowa.edu.

The University of Iowa is an Equal Opportunity/Affirmative Action Employer. Women and minorities are strongly encouraged to apply.



SOOCHOW UNIVERSITY

Faculty Positions

The Soochow University, founded in 1900 in Suzhou, Jiangsu, is a premier university in China. The University is committed to excellence in education, science and innovation. A major expansion of its research programs is underway across a broad spectrum of areas in mathematics, physics, chemistry, mechanical and electronic engineering, optical and energy engineering, material and computer sciences, urban planning, environmental science, textile engineering and design, life sciences, and medicine. A complete listing of open positions is available at <http://rsc.suda.edu.cn/zpxx.asp?type=15>.

We seek distinguished professors, academic pacesetters and laboratory heads to join us at the **Institute of Neuroscience**. Successful candidates should have a Ph.D. degree with more than 3 years of research experience abroad and an outstanding record of academic achievements with high quality publications in top international journals. We provide competitive relocation, salary packages, generous start-up funds and state-of-the-art facilities.

Interested individuals should send curriculum vitae, a summary of accomplishments, future research plans, and a list of three references to LI Jun, Institute of Neuroscience, Soochow University, email: lijun860227@hotmail.com, campus of Soochow University, 199 Ren-Ai Road, Suzhou, Jiangsu, 215123, China, <http://www.suda.edu.cn/>.

SCIENCE Editorial

Science and AAAS seek a talented scientist to serve as an **Associate Editor** for our new interdisciplinary journal, *Science Translational Medicine*.

This position is designed for an individual with broad interests, a lively curiosity, and experience with cutting-edge research in at least one, but preferably more than one, biomedical or clinical research field.

The tasks include, but are not limited to:

- Manage the review, selection, and editing of submitted manuscripts;
- Judge the scientific value of research;
- Foster relationships and communication with the scientific community through literature reviews, meetings and professional contacts;
- Select reviewers for submitted manuscripts;
- Discuss and make recommendations regarding manuscripts and reviews with other staff, advisers, authors;
- Write summaries of research results for publication;
- Guide authors on manuscript revisions;
- Edit the manuscripts for scientific content and style before and after revisions;
- Follow the manuscript through production process to ensure material is published in a timely manner;
- Travel to scientific meetings.

The minimum qualifications to be competitive and considered for the position are:

- Mastery of a professional field typically acquired through completion of a doctoral degree in at least one biomedical or clinical research field;
- 3-5 years experience, including post doctoral research experience and multiple publications;
- Ability to work constructively as a member of a team;
- Experience with cutting-edge research in one of the fields mentioned above;
- Comprehensive knowledge of scientific research methods in order to discuss technical issues with authors;
- Exceptional writing, communication, and listening skills in order to communicate with authors and reviewers in evaluating, editing and modifying manuscripts.

Previous editorial experience is not required.

If you would like to be a member of the AAAS team, please visit our Job Information website at <http://www.aaas.org/careercenter/employmentataaas/> to get more information and to apply online today.

**Faculty Positions in
Bioinformatics and
Computational Biology**
at the Jan and Dan Duncan
Neurological Research Institute



Texas Children's Hospital and **Baylor College of Medicine** are seeking several outstanding computational biologists to join the faculty of the **Jan and Dan Duncan Neurological Research Institute (NRI)**. The NRI will bring together world experts in neurology, neuroscience, pediatrics, genetics, physiology, developmental biology, chemistry, behavior, imaging and applied mathematics to pursue collaborative, interdisciplinary basic and translational research on a variety of neurological and neurodevelopmental disorders. The new 13-story NRI, is nestled between Baylor College of Medicine, Texas Children's Hospital and M.D. Anderson Cancer Center and will include state-of-the-art computational resources as well as core facilities overseen by experts in imaging, physiology, neurobehavior, neuropathology and animal models of human disease among others.

The successful faculty will have appointments in the Departments of Pediatrics and other basic science departments (according to interest and expertise) at Baylor College of Medicine; joint appointments at Rice University will be considered when appropriate. The primary responsibility will be to establish a cutting-edge Bioinformatics and Computational Biology Program within the NRI that will promote the research interests of the appointees as well as foster collaborations with other NRI faculty members. We seek exceptional research scientists that can capitalize on our highly collaborative environment. We envision the formation of interdisciplinary teams of investigators focused on unraveling the mechanisms responsible for brain disorders using systems approaches. The candidates should complement existing strengths at the NRI with their expertise in quantitative analysis such as statistical pattern recognition, machine learning, data mining, neuroinformatics, and algorithm optimization, and will be expected to contribute to the goals of the Institute by developing methods for high-throughput image analysis and for integrating 'omics' data. A generous start-up package and state-of-the-art computational resources will be provided.

Candidates must possess a Ph.D., M.D. or equivalents in Neuroscience, Applied Mathematics, Computational Biology, or Biophysics etc. Academic rank will be commensurate with experience. Interested individuals should send a curriculum vitae and personal statement of their professional goals to:

**Huda Y. Zoghbi, M.D.,
Director, Neurological Research Institute;
Baylor College of Medicine
One Baylor Plaza, MS
BCM225, Room T807
Houston, TX 77030.**

Baylor College of Medicine is an Equal Opportunity, Affirmative Action, Equal Access Employer.



Director of Proteomics and Mass Spectrometry University of Massachusetts Medical School

The University of Massachusetts Medical School seeks an outstanding individual to serve as Director of its Proteomics Facility. Over the past decade, the medical school has expanded its research program by establishing new departments and programs, by bringing over 120 new faculty to the campus and by constructing state-of-the-art facilities. The next growth phase will focus on translational research including RNA therapeutics, stem cell biology and gene therapy, and will be housed in an adjacent building now under construction. Exciting opportunities in proteomics for the existing and developing programs require a highly qualified individual to lead the Proteomics Facility.

Competitive candidates will have a PhD and at least five years of professional experience in the application of a variety of sophisticated mass spectrometric methods to biology and/or medicine. Candidates will also have demonstrated leadership abilities critical for managing a multi-component facility and its staff. The individual will be responsible for training students and postdoctoral fellows, establishing collaborations with our faculty, and advancing the capabilities of the facility via competitive funding from external sources. Information on our current proteomics facilities, which will be combined into an integrated facility under the leadership of the new director, can be found under Core Facilities at <http://www.umassmed.edu/research>. Applicants should submit a cover letter explaining their qualifications and their interest in this position, a curriculum vitae and the names and contact information for three individuals who could provide letters of recommendation to <http://www.academicjobsonline.org>. Minorities and women are especially encouraged to apply. Inquiries but not application materials may be addressed to Karen.Welch@umassmed.edu.

As an Equal Opportunity and Affirmative Action Employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives and backgrounds.



Assistant Professor (tenure-track) Faculty Positions Department of Biochemistry, School of Medicine West Virginia University Morgantown, WV

The Department of Biochemistry is expanding its faculty with positions at the Assistant Professor rank (tenure-track) in the area of Cancer Biology. The department has existing strengths in the areas of regulation of gene or protein expression, analysis of oncogene signaling networks, metabolism, and mechanisms of cell death by apoptosis or anoikis. Investigators who complement these strengths, or with research programs in emerging areas of interest are especially encouraged to apply. Innovative programs addressing fundamental questions related to cancer will be given priority. Minimal qualifications are a PhD plus three years of highly productive postdoctoral research. The successful candidate will be expected to develop an extramurally funded research program, demonstrate excellence in the departmental teaching mission, and be an active participant in department and school-wide research forums and seminars. West Virginia University is a comprehensive, land grant university with 185 undergraduate, graduate and professional programs and an enrollment of 28,840 students, including 6,910 professional and graduate students. The Robert C. Byrd Health Sciences Center includes the Schools of Medicine, Pharmacy, Dentistry and Nursing. Recent expansion at the Health Sciences Center includes two new research buildings, which collectively provide approximately 200,000 sq. ft. of additional research space, to accommodate our growth in research programs and core facilities. With a metro area population of over 115,000, Morgantown is consistently rated as one of the best small cities in the U.S., with an affordable cost of living, excellent schools, ample job opportunities, a picturesque countryside, and many outdoor recreational opportunities. Review of applications will begin immediately and continue until the position is filled.

Please submit as a single electronic file your C.V., summary of past research accomplishments and future research plans, and the names of three references to: Ms. Lana Yoho, lyoho@hsc.wvu.edu.

WVU is an Equal Opportunity/Affirmative Action Employer.



National Institute for Basic Biology, Okazaki, Japan

Three Professorships

The National Institute for Basic Biology (NIBB), Okazaki, Japan, invites applications for three professorships.

Since its establishment following a recommendation by the Science Council of Japan in 1977, NIBB has conducted research activities as a center of excellence in biological sciences. The research activities at NIBB are among the highest ranked in the field of biological sciences in Japan, as judged by factors such as citation index per paper. To continue our efforts to serve as a national center for biological sciences, NIBB recruits leading scientists not only from Japan but also from abroad.

We seek candidates with excellent research records who can be international leaders in the field of basic biology. In particular, those using biological approaches to answer fundamental questions in such fields as (1) Neuroscience, (2) Plant science, and (3) System sciences for environmental responses of biological organisms. In addition, we welcome applications from candidates of any field that have excellent potential to advance basic biology.

To apply, please see the website below for a list of required documents; which must be received no later than **January 18, 2010**. Online application is possible for overseas applicants. For more information, please visit the website at <http://www.nibb.ac.jp/profapplication/>

Inquires may be directed to Professor Tetsuo Yamamori, National Institute for Basic Biology, 38 Myodaiji, Okazaki, 444-8585, Japan, Tel/ Fax: 81-564-55-7615, E-mail: profapl-inquiry@nibb.ac.jp



SENIOR POSITION IN HUMAN AND MOLECULAR GENETICS (HMG) VCU INSTITUTE OF MOLECULAR MEDICINE (VIMM) AND VCU MASSEY CANCER CENTER (MCC)

VIRGINIA COMMONWEALTH UNIVERSITY (VCU) SCHOOL OF MEDICINE, HMG, VIMM and MCC

Under the leadership of Dr. Paul B. Fisher the **Department of Human and Molecular Genetics (HMG)** and the **VCU Institute of Molecular Medicine (VIMM)** in collaboration with the **VCU Massey Cancer Center (MCC)** in Richmond, Virginia seeks to recruit a seasoned investigator who focuses on cancer development and progression and whose research bridges the gap between laboratory discovery and clinical trials. Research that employs current genomic discoveries in medicine with an emphasis on translating these findings into improved approaches for diagnosis and treatment of neoplastic diseases are areas of high priority of this institutional initiative.

The ideal candidate will be an experienced investigator who has demonstrated consistent research excellence, with a sustained track record of research funding, high-level publications and administrative experience. We are particularly interested in candidates that have managed multifaceted, interactive research programs using hypothesis-based, innovative approaches to address important health-related areas. Candidates with a sustained record of NIH funding will be given the highest priority. The appropriate candidate will be recruited at the level of Associate/Full Professor with qualifications commensurate with tenure. This individual will play a major role in VCU SOM and will be considered for appointment to a senior administrative position in HMG. An appropriate candidate will also be considered for appointment as the Associate Director of the VIMM and/or Co-Program Leader in the MCC.

HMG, VIMM and MCC provide an interactive and collaborative research and educational environment that will facilitate the training of the next generation of research scientists, clinicians and academicians, and will provide a direct conduit for the translation of genetic information from bench-to-bedside. Outstanding state-of-the-art core research facilities with a generous start-up and support package available for the qualified applicant.

Richmond, VA provides an ideal rural living and cultural environment with affordable housing, outstanding school systems and ready access to other metropolitan areas (including Washington, DC, Baltimore, Philadelphia and New York). Moreover, the City of Richmond and surrounding areas offer a diverse and rich cultural heritage that engenders a high quality of living for its residents.

Interested candidates should provide by e-mail (preferably as a single PDF file): a letter of interest, a curriculum vitae, a description of administrative philosophy, and an outline of research interests and future research directions, with contact information for three to four references to:

Dr. Paul B. Fisher (hmgvimm@vcu.edu)

**Department of Human and Molecular Genetics, Virginia Commonwealth University, School of Medicine
1101 East Marshall Street, Sanger Hall Building, Room 11-015, Richmond, VA 23298-0033**

Review of Applications will begin **February 1, 2010** and will continue till the position is filled.

VCU is an EEO/AA Employer: Female, Minorities and persons with disabilities are encouraged to apply.



DEAN, COLLEGE OF NATURAL RESOURCES

MOSCOW, IDAHO

The University of Idaho, a leading research institution and land-grant university, seeks as its next dean of the College of Natural Resources, an experienced executive and research professional who will provide strategic and operational leadership. The university recently has implemented a strategic action plan to guide its ongoing renewal and expansion for the future and is poised to develop the next phase of strategic innovation (<http://www.uidaho.edu/provost/strategicactionplan.aspx>).

The dean is the chief administrative officer of the college and reports to the provost and executive vice president. The dean has authority and responsibility for all aspects of curriculum planning and development, faculty and staff evaluation and development, budget and facilities.

Minimum Qualifications: The candidate must possess an earned doctorate/terminal degree from an accredited institution and credentials to hold a tenured professorship.

Completed applications should include a letter of interest and qualifications relative to the position description, statements on the visions for education and research appropriate for the college of a land grant university, vitae, and contact information for five references (including names, title, address, phone number and email address). Nominations are welcome. All applications will be given full consideration. Review of applications will begin February 1, 2010 and the search process will continue until an appointment is made. Search Chairperson is Dean Scott Wood, University of Idaho, College of Science, P.O. Box 443025, Moscow, Idaho 83844-3025. Electronic online application: <http://www.hr.uidaho.edu>.

An Equal Opportunity/Affirmative Action Employer.



UNIVERSITY OF VIRGINIA HEALTH SYSTEM
Center for Membrane Biology in the Department of Molecular Physiology and Biological Physics, is seeking to fill two **tenure-track faculty positions**. Rank will depend on qualifications. Candidates must have a PhD or MD degree, at least two years of postdoctoral experience,

and are expected to be competitive at a national level by recognition through peer-reviewed publications and demonstrated ability to secure competitive national grant support. The Center housed in newly constructed space is based in the Department of Molecular Physiology and Biological Physics in the School of Medicine and also draws faculty from several other Departments.

UVa has a history of outstanding research in the structure and function of membranes, and current strengths include (1) the structural biology of membrane channels, transporters and receptors, (2) signal transduction in membranes, (3) viral and intracellular membrane fusion, (4) trafficking of membrane proteins, (5) cell adhesion, and (6) lipid-protein interactions. We especially encourage applicants with expertise that complements and broadens these areas, including translational research more closely related to disease. The positions offer access to state-of-the-art facilities for membrane protein production, structure determination by crystallography, NMR spectroscopy, and electron microscopy, as well as other biophysical techniques. In addition to membrane biology, the Department of Molecular Physiology and Biological Physics also has strengths in cardiovascular physiology, cancer biology, and structural biology in general.

To apply, visit <https://jobs.virginia.edu> and search on **Posting Number 0604829**, complete a Candidate Profile online and attach Curriculum Vitae with publication list, cover letter, a statement of significant research accomplishments and future research plans, and a list of three references with name, email address and phone number. Review of applications will begin **January 25, 2010** and the positions will remain open to applications until filled.

For additional information about the positions, please contact the chair of the search committee, **Dr. Lukas K. Tamm, Director of the Center for Membrane Biology, Department of Molecular Physiology and Biological Physics, University of Virginia Health System, P.O. Box 800886, Charlottesville, VA 22908-0886**. For further information about the application process, please contact **Howard Phipps** at memsearch@virginia.edu. We also recommend you send a pdf copy of your application to this email address.

The University of Virginia is an Equal Opportunity/Affirmative Action Employer.

march 8-9, 2010

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individualized medical care

economics

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AWARDS

ROBERT J. AND CLAIRE PASAROW FOUNDATION 23rd Annual MEDICAL RESEARCH AWARDS CALL FOR NOMINATIONS Cancer, Cardiovascular Disease, Neuropsychiatry

The Foundation has established three yearly medical prizes for distinguished accomplishment in research in order to increase public awareness of vital areas of investigation. This is the twenty-third year of the awards program. Each award is for \$50,000, presented directly to the awardee. The three prizes – one in each of the three fields – are given for extraordinary basic and/or clinical or translational research.

Cancer: including basic cellular processes and the various forms of cancer. **Past awardees:** Peter K. Vogt, PhD; Irving L. Weissman, MD; George F. Vande Woude, PhD; Erkki Ruoslahti, MD; Harold N. Weintraub, MD, PhD; Ronald M. Evans, PhD; Stanley J. Korsmeyer, MD; Carlo M. Croce, MD; Alfred G. Knudson, Jr., MD, PhD; Robert A. Weinberg, MD; Eric S. Lander, D.Phil.; Paul L. Modrich, PhD; Anthony S. Fauci, MD; Alexander J. Varshavsky, PhD; Tom Maniatis, PhD; Roger D. Kornberg, PhD; Elizabeth H. Blackburn, PhD; Fred W. Alt, PhD; Bert O'Malley, MD; Tony Hunter, PhD; and Bert Vogelstein, MD

Cardiovascular Disease: including disorders of the heart and vascular system. **Past awardees:** Burton E. Sobel, MD; Harvey Feigenbaum, MD; Bernardo Nadal-Ginard, MD, PhD; Mordecai P. Blaustein, MD; Jonathan Seidman, PhD and Christine Seidman, MD; Glenn A. Langer, MD; Philip Majerus, MD; Jan L. Breslow, MD; Kenneth R. Chien, MD, PhD; Michael A. Gimbrone, Jr., MD; Masashi Yanagisawa, MD, PhD; Mark T. Keating, MD; Eric N. Olson, PhD; Richard P. Lifton, MD, PhD; Robert J. Lefkowitz, MD; Shaun Coughlin, MD, PhD; Judah Folkman, MD; Barry S. Collier, MD; Douglas C. Wallace, PhD; Daniel Steinberg, MD, PhD; and Richard O. Hynes, PhD

Neuropsychiatry: including neurologic and mental disorders. **Past awardees:** Nancy Wexler, PhD; Eric R. Kandel, MD; Floyd E. Bloom, MD; Solomon H. Snyder, MD; Michael E. Phelps, PhD; Patricia S. Goldman-Rakic, PhD; Huda Akil, PhD and Stanley Watson, MD, PhD; Arvid Carlsson, MD, PhD and Philip Seeman, MD, PhD; Stanley B. Prusiner, MD; Joseph T. Coyle, MD; Eric J. Nestler, MD, PhD; Fred H. Gage, PhD; Michael I. Posner, PhD and Marcus E. Raichle, MD; Pasko Rakic, MD, PhD; Seymour Benzer, PhD; Tomas Hökfelt, MD, PhD; Thomas M. Jessell, PhD; Judith L. Rapoport, MD; Bruce McEwen, PhD; Huda Y. Zoghbi, MD; and Aaron T. Beck, MD

The criterion for the Pasarow Medical Research Awards is evidence of extraordinary accomplishment in medical science.

Nominators for the 23rd Award should provide a letter of no more than one page stating the rationale for the nomination and a copy of the nominee's curriculum vitae and bibliography in two-page NIH format. Applications will be reviewed by the Board of Directors in consultation with various medical scholars. Members of the Board of Directors are **Michael Pasarow**, Chairman; **Anthony H. Pasarow**, Co-Treasurer; **Susan A. Pasarow, MSW**, Co-Treasurer; **Jack D. Barchas, MD**, President; **Shaun Coughlin, MD, PhD** – University of California, San Francisco; **Ronald M. Evans, PhD** – The Salk Institute; **Brian E. Henderson, MD** – University of Southern California; **Joseph P. Van Der Meulen, MD** – University of Southern California, and **Alexander J. Varshavsky, PhD** – CalTech.

Nominations should be sent to: **Robert J. and Claire Pasarow Foundation, c/o Jack D. Barchas, MD; Weill Cornell Medical College, 1300 York Avenue, Box 171, Room F-1231, New York, NY 10065.**

For more information, please contact **Jack D. Barchas, MD** at (212) 746-3770 or jbarchas@med.cornell.edu. Nominations should be received by **Monday, February 10, 2010.**

The Pasarow Foundation congratulates two past Pasarow award winners for their stunning achievements in 2009:

Elizabeth H. Blackburn, PhD (recipient of the 17th Pasarow Award in Cancer Research) - Along with Drs. Carol W. Greider and Jack W. Szostak, recipient of **The Nobel Prize in Physiology or Medicine 2009** for their fundamental research which led to the discovery of "how chromosomes are protected by telomeres and the enzyme telomerase."

Michael I. Posner, PhD (recipient of the 13th Pasarow Award in Neuropsychiatry with Marcus Raichle, MD) – received the **2009 President's National Medal of Science** "for incisive and accurate modeling of functional tasks, and his development of methodical and conceptual tools to help understand the mind and the development of brain networks of attention."

POSITIONS OPEN



Marshall University is seeking a tenure-track **ASSISTANT PROFESSOR** of vertebrate evolutionary biology in the Department of Biological Sciences, College of Science. Required qualifications include a Ph.D. in biology, paleontology or related discipline; relevant postdoctoral experience; experience teaching human anatomy; and a record of research productivity. Demonstrated excellence in teaching and outstanding communication skills are desired.

The successful candidate will participate in undergraduate and graduate (M.S./M.A.) teaching and research mentorship, and will be expected to establish an active, externally funded research program. The primary teaching responsibility will be a service course in human anatomy without cadaver dissection. We also seek a colleague who will support our interdisciplinary programs and contribute to the university's general education curriculum with its emphasis on First-Year Seminar and core courses, which are intended to enhance students' critical thinking. The desired area of research is broadly defined as vertebrate evolutionary biology, but should complement existing departmental strengths in vertebrate paleobiology, paleontology, mammalogy, and herpetology. Outstanding applications from other related fields will also be considered.

Interested applicants should send cover letter, current curriculum vitae, statements of research interests and teaching philosophy, selected electronic reprints, university transcripts (official transcripts will be requested of finalists), and request three letters of reference sent to: **e-mail: biologysearch@marshall.edu**. Please assemble all documents, except letters of reference, into a single PDF file. Electronic submission is preferred, but paper application materials may be sent to: **Dr. F. Robin O'Keefe, Department of Biological Sciences, Marshall University, One John Marshall Drive, Huntington, WV 25755**.

Application deadline: February 15, 2010. *Marshall University is an Affirmative Action Equal Opportunity Employer dedicated to increasing the diversity of its faculty and students.*

ASSISTANT PROFESSOR — BIOLOGY

Saint Francis University invites applications for an Assistant Professor to begin August 2010. The candidate should hold a Ph.D. in physiology or related field.

Applicants must have a strong commitment to undergraduate education and research. Preference will be given to candidates with teaching experience. The selected applicant is expected to teach courses in human anatomy and physiology, vertebrate physiology, introductory biology, and other courses relating to candidate's background.

Candidates should send their curriculum vitae, undergraduate and graduate transcripts, three letters of recommendation, statements of teaching philosophy and research interests to: **Search Committee for Assistant Professor of Biology, c/o Office of Human Resources, Saint Francis University, P.O. Box 600, Loretto, PA 15940**. E-mail: **positions@francis.edu**.

Review of applicants will begin January 8, 2010. *Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL RESEARCHER POSITIONS

in Cardiovascular Disease and Tuberculosis

Candidates with molecular, cell biological, or biochemical skills needed to work on problems relevant to tuberculosis and cardiovascular disease at the Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida. Send curriculum vitae and e-mail addresses of at least three references to **P.E. Kolattukudy, e-mail: pk@mail.ucf.edu**.

The University of Central Florida is an Equal Opportunity, Equal Access, and Affirmative Action Employer. As a member of the Florida State University System, all application materials and selection procedures are available for public review.

POSITIONS OPEN



MEDICINAL CHEMIST

The University of Tennessee College of Pharmacy has a 12-month, tenure-track (**ASSISTANT, ASSOCIATE, or FULL PROFESSOR**) position available. Candidates must have a Ph.D. degree in medicinal chemistry or a related area and an externally funded research program. The candidate must have an interest in teaching medicinal chemistry at the Pharm.D. and graduate levels and possess good communication skills. All areas of medicinal chemistry and related disciplines will be considered, but preference will be given to candidates with research programs in chemical biology and anti-infective agents. The position offers startup funds, an attractive research incentive plan, and a competitive salary and benefits package commensurate with experience. The College will be moving into a new building (183,857 gross square feet) in 2010. Applications are being accepted. Applicants should send a letter, including a summary of future research plans, curriculum vitae, and three letters of reference to: **Isaac Donkor, Ph.D., Professor and Vice Chair, Department of Pharmaceutical Sciences, 847 Monroe Avenue, Suite 327, Memphis, TN 38163**. Applications will be accepted until the position is filled. *The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX/Sec. 504/ADA/ADEA Employer.*



TEXAS A&M
HEALTH SCIENCE CENTER
COLLEGE OF MEDICINE

VACCINE DEVELOPMENT AND BACTERIAL PATHOGENESIS

POSTDOCTORAL FELLOW position is available immediately to join the research group of **Dr. James E. Samuel** studying *Coxiella burnetii*. Individual will be primarily responsible for conducting collaborative research on vaccine development and publication of results. Research will emphasize the immune response to protective antigens in Q fever using mouse and guinea pig virulence models. Additional opportunities for investigation include novel genetic approaches for molecular pathogenesis with the agent. Ph.D. required and a record of productive experience in immunology, animal models, and/or with bacterial pathogens preferred. Send curriculum vitae and names and addresses of three references to: **Dr. James E. Samuel, Department Microbial and Molecular Pathogenesis, Texas A&M Health Science Center, MS 1114, 407 Reynolds Medical Building, College Station, TX 77843-1114**. Fax: 979-845-3479; e-mail: **jsamuel@medicine.tamhsc.edu**. Contact **Dr. Samuel, telephone: 979-862-1684** for additional information. *The Texas A&M Health Science Center is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN



Washington State University Tri-Cities invites applications for a full-time, tenure-track **ASSISTANT PROFESSOR** position in applied microbiology with an appointment expected in the School of Molecular Biosciences at WSU. The successful candidate will be expected to teach at the undergraduate and graduate levels and should have a background that will allow broad participation in the teaching program. Expected research contributions in applied microbiology are summarized below. We encourage applications from individuals with demonstrated expertise, as evidenced by peer-reviewed publications in the fields of cellular and molecular microbiology (e.g., applied, environmental, pathogenic). Preference will be given to applicants whose research complements current research strengths within the Center for Bioproducts and Bioenergy and to the university's Viticulture and Enology program. The campus also has a strong partnership with the Pacific Northwest National Laboratory. A competitive candidate will have a Ph.D. in microbiology or a closely related field, ability to teach undergraduate and graduate courses, expertise in applied microbiology, a record of research productivity with the potential to obtain extramural support, and ability to work with multidisciplinary teams. Additional information is available for **position #5272** at **website: http://www.wsujobs.com**. Interested individuals should submit electronically: (1) a cover letter discussing training and experience as related to the required and desired qualifications; (2) curriculum vitae; and (3) the names and contact information for three references. Review of completed applications will begin on January 29, 2010. Applications should be submitted to: **Microbiology Search, c/o Bonnie Bates (e-mail: bbates@tricity.wsu.edu), Washington State University Tri-Cities, 2710 University Drive, Richland, WA 99354**.

Washington State University is an Equal Opportunity/Affirmative Action Educator and Employer. Members of groups underrepresented in science are encouraged to apply.

POSTDOCTORAL FELLOWSHIP

The Lillehei Heart Institute is a premier institute at the University of Minnesota, Minneapolis with state-of-the-art technologies and core facilities focused on the molecular regulation of myogenesis, stem cell and regenerative biology. We are accepting applications for highly motivated Postdoctoral Fellows to work on NIH-funded research pertaining to the role of transcription factor mediated networks to direct the fate of stem and iPS cells to a mesodermal fate (i.e., cardiac, endothelial, skeletal muscle). Ph.D. and expertise with molecular biological-biochemistry techniques, the use of transgenesis and knockout technologies will be required for this position. Interested applicants should apply online to: **Daniel J. Garry, M.D., Ph.D., Director of Lillehei Heart Institute, University of Minnesota** at **website: http://employment.umn.edu** (reference **job search #161786**) and include curriculum vitae, statement of interest, and names of three references.

The University of Minnesota is an Affirmative Action/Equal Opportunity Employer.

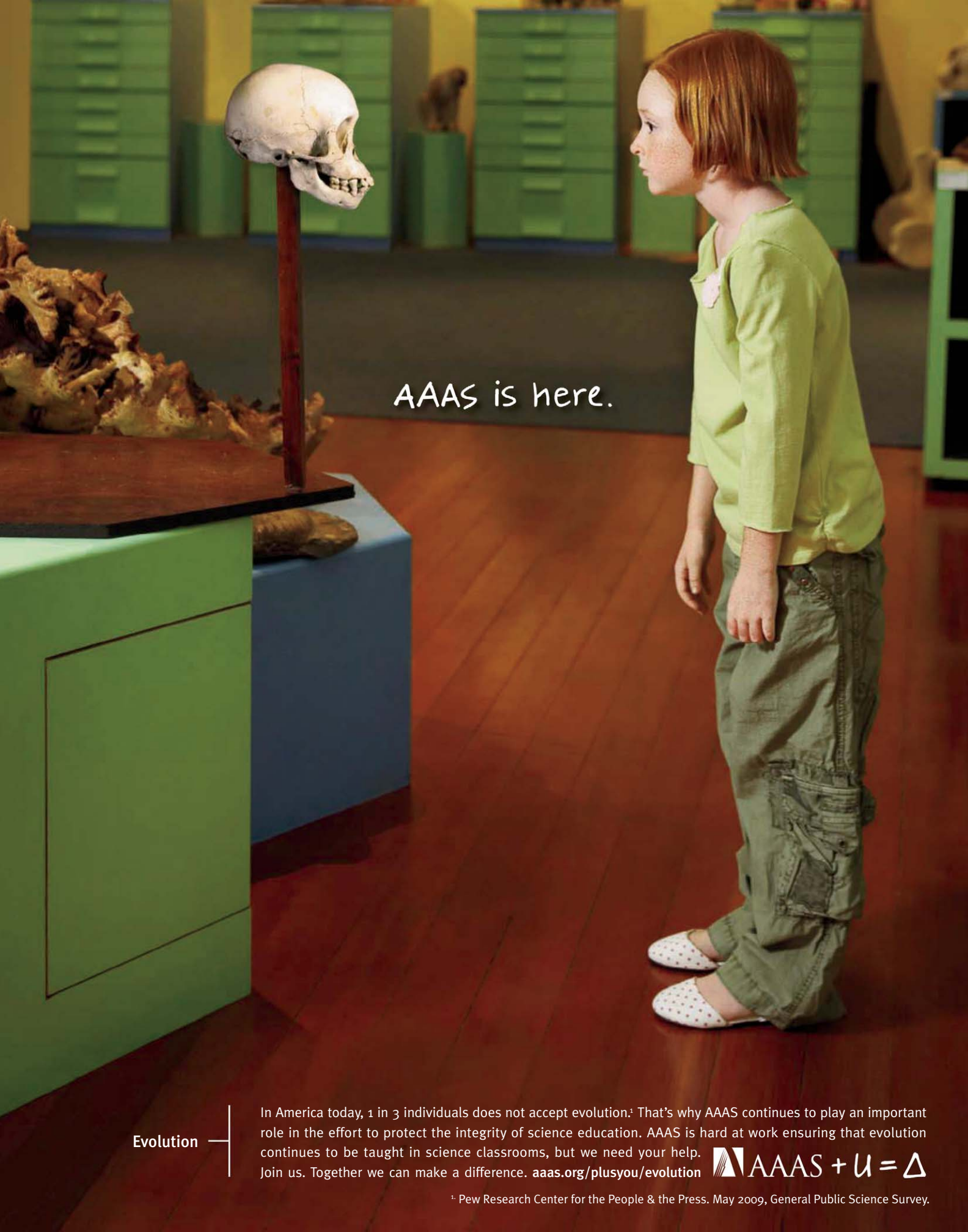
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AAAS is here.

Evolution

In America today, 1 in 3 individuals does not accept evolution.¹ That's why AAAS continues to play an important role in the effort to protect the integrity of science education. AAAS is hard at work ensuring that evolution continues to be taught in science classrooms, but we need your help. Join us. Together we can make a difference. aaas.org/plusyou/evolution



¹ Pew Research Center for the People & the Press. May 2009, General Public Science Survey.